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Genome wide search for genetic determinants of habitual alcohol, tobacco and coffee use, obesity-related traits, response to mental and physical stress and hemodynamic traits

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Cette thèse intitulée:

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Résumé

Les habitudes de consommation de substances psychoactives, le stress, l'obésité et les traits cardiovasculaires associés seraient en partie reliés aux mêmes facteurs génétiques. Afin d'explorer cette hypothèse, nous avons effectué, chez 119 familles multi-générationnelles québécoises de la région du Saguenay-Lac-St-Jean, des études d'association et de liaison pangénomiques pour les composantes génétiques : de la consommation usuelle d'alcool, de tabac et de café, de la réponse au stress physique et psychologique, des traits anthropométriques reliés à l'obésité, ainsi que des mesures du rythme cardiaque (RC) et de la pression artérielle (PA). 58000 SNP*s* et 437 marqueurs microsatellites ont été utilisés et l'annotation fonctionnelle des gènes candidats identifiés a ensuite été réalisée.

Nous avons détecté des corrélations phénotypiques significatives entre les substances psychoactives, le stress, l'obésité et les traits hémodynamiques. Par exemple, les consommateurs d'alcool et de tabac ont montré un RC significativement diminué en réponse au stress psychologique. De plus, les consommateurs de tabac avaient des PA plus basses que les non-consommateurs. Aussi, les hypertendus présentaient des RC et PA systoliques accrus en réponse au stress psychologique et un indice de masse corporelle (IMC) élevé, comparativement aux normotendus. D'autre part, l'utilisation de tabac augmenterait les taux corporels d'épinéphrine, et des niveaux élevés d'épinéphrine ont été associés à des IMC diminués. Ainsi, en accord avec les corrélations inter-phénotypiques, nous avons identifié plusieurs gènes associés/liés à la consommation de substances psychoactives, à la réponse au stress physique et psychologique, aux traits reliés à l'obésité et aux traits hémodynamiques incluant CAMK4, CNTN4, DLG2, DAG1, FHIT, GRID2, ITPR2, NOVA1, NRG3 et PRKCE. Ces gènes codent pour des protéines constituant un réseau d'interactions, impliquées dans la plasticité synaptique, et hautement exprimées dans le cerveau et ses tissus associés. De plus, l'analyse des sentiers de signalisation pour les gènes identifiés (P = 0,03) a révélé une induction de mécanismes de *Potentialisation à Long Terme*.

Les variations des traits étudiés seraient en grande partie liées au sexe et au statut d'hypertension. Pour la consommation de tabac, nous avons noté que le degré et le sens des corrélations avec l'obésité, les traits hémodynamiques et le stress sont spécifiques au sexe et à la pression artérielle. Par exemple, si des variations ont été détectées entre les hommes fumeurs et non-fumeurs (anciens et jamais), aucune différence n'a été observée chez les femmes. Nous avons aussi identifié de nombreux traits reliés à l'obésité dont la corrélation avec la consommation de tabac apparaît essentiellement plus liée à des facteurs génétiques qu'au fait de fumer en lui-même. Pour le sexe et l'hypertension, des différences dans l'héritabilité de nombreux traits ont également été observées. En effet, des analyses génétiques sur des sous-groupes spécifiques ont révélé des gènes additionnels partageant des fonctions synaptiques : CAMK4, CNTN5, DNM3, KCNAB1 (spécifique à l'hypertension), CNTN4, DNM3, FHIT, ITPR1 and NRXN3 (spécifique au sexe). Ces gènes codent pour des protéines interagissant avec les protéines de gènes détectés dans l'analyse générale. De plus, pour les gènes des sous-groupes, les résultats des analyses des sentiers de signalisation et des profils d'expression des gènes ont montré des caractéristiques similaires à celles de l'analyse générale.

La convergence substantielle entre les déterminants génétiques des substances psychoactives, du stress, de l'obésité et des traits hémodynamiques soutiennent la notion selon laquelle les variations génétiques des voies de plasticité synaptique constitueraient une interface commune avec les différences génétiques liées au sexe et à l'hypertension. Nous pensons, également, que la plasticité synaptique interviendrait dans de nombreux phénotypes complexes influencés par le mode de vie. En définitive, ces résultats indiquent que des approches basées sur des sous-groupes et des réseaux amélioreraient la compréhension de la nature polygénique des phénotypes complexes, et des processus moléculaires communs qui les définissent.

Mots-clés : cartographie génique, substances psychoactives, traits reliés à l'obésité, stress psychologique, stress physique, rythme cardiaque, pression artérielle, synapse, analyse de sous-groupes, réseau de gènes, réseau de phénotypes.

Abstract

Links among substance use, obesity, stress and related cardiovascular outcomes may be in part due to shared genetic factors. To investigate this hypothesis, we performed genome-wide linkage and association scans for genetic components of habitual alcohol, tobacco and coffee use, response to mental and physical stress, obesity related anthropometric traits and heart rate (HR) and blood pressure (BP) measurements in 119 multigenerational French Canadian families from founder population of Saguenay-Lac-St-Jean region using 58000 SNPs and 437 microsatellite markers and followed with functional annotation on resulted genes.

We found significant phenotypic correlations among substance use, obesity, stress and hemodynamic traits. For instance, alcohol and tobacco users had attenuated HR response to mental stress; moreover, tobacco users had lower BP compared to non users; Hypertensives had stronger HR and systolic blood pressure (SBP) response to mental stress and higher body mass index (BMI), compared to normotensives; Use of tobacco seemed to increase the epinephrine level in body and higher epinephrine level was correlated with lower BMI. Consistent with phenotypic relatedness, we found numerous shared genes associated / linked to substance use, obesity-related traits, response to mental and physical stress and hemodynamic traits including CAMK4, CNTN4, DLG2, DAG1, FHIT, GRID2, ITPR2, NOVA1, NRG3 and PRKCE forming protein interaction network, involved in synaptic plasticity and highly expressed in brain related tissues; moreover, pathway analysis on identified genes pointed (P = 0.03) to *Long-Term Potentiation pathway*.

Large portions of variation of studied traits were explained by sex and hypertension status, focusing on tobacco use we noted that degree and the direction of correlations of obesity, hemodynamic and stress related traits with tobacco use vary according to sex and hypertension status; for instance, while in males, current tobacco users were slender compared to never or former tobacco users, there were no such differences in females; moreover, we found several obesity related traits that their correlations with smoking behavior seemingly root in genetic factors rather than smoking effect itself. Sex- and hypertension differences in heritabilities of many of these traits were also observed;

meanwhile, specific subgroup genetic analyses uncovered additional shared synaptic genes among these traits including CAMK4, CNTN5, DNM3, KCNAB1 (Hypertension-specific), CNTN4, DNM3, FHIT, ITPR1 and NRXN3 (Sex-specific) having protein interactions with genes driven from general analysis; moreover, the results of pathway analysis and reported gene expression profiles of resulted genes from subgroup analyses revealed similar characteristics to those from general analysis.

The substantial overlap among genomic determinants of substance use, stress, obesity and hemodynamic traits supports the notion that the genetic variations in pathways of synaptic plasticity may be a common interface behind them as well as observed sex and hypertension genetic differences, we also think synaptic plasticity may underlie many complex phenotypes in which life style is a contributing factor; moreover, our findings indicate considering subgroup and network-based approaches enhance understanding of polygenic nature of complex phenotypes as well as shared molecular underpinnings among them.

Keywords : genetic mapping, substance use, obesity-related traits, mental stress, physical stress, heart rate, blood pressure, synapse, subgroup analysis, gene network, phenotype network

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Introduction

Article1

Liste des sigles et abréviations

ACTH: adrenocorticotropic hormone

Asn: asparagine

Asp: aspartic acid

BMI: body mass index

BP: blood pressure

cAMP: cyclic adenosine monophosphate

CBG: corticosteroid-binding globulin

Chr: chromosome

CHUM: centre hospitalier de l'université de montréal

cM: centi morgan

CNS: central nervous system

CRF: corticotropin releasing factor

DBP: diastolic blood pressure

EP: epinephrine

FBAT: family-based association test

GABA: gama-aminobutyric acid

GEE: generalized estimating equation

GnRH: gonadotropin-releasing hormone

GWAS: genome-wide association study

H2r: heritability

HPA: hypothalamic-pituitary-adrenal

HR: heart rate

HSP: heat shock protein

HT: hypertension

HWE: hardy-weinberg equilibrium

IBD: identity-by-descent

IP3: inositol trisphosphate

km: kilometer

LD: linkage disequilibrium

LDL: low-density lipoprotein

LOD: logarithm of odds

LTP: long-term potentiation pathway

MAF: minor allele frequency

MAP: mean arterial pressure

MAPK: mitogen-activated protein kinase

N: number

NAc: nucleus accumbens

NE: norepinephrine

NIH: national institutes of health

NPL: non-parametric linkage

NPY: neuropeptide y

OMIM: online mendelian inheritance in man

P: p-value

POMC: proopiomelanocortin

PP: pulse pressure

QTL: quantitative trait loci

RAAS: renin-angiotensin-aldosterone system

SBP: systolic blood pressure

SE: standard error

SLSJ: saguenay-lac-st-jean

SNP: single-nucleotide polymorphism

SNS: sympathetic nervous system

T1D: type 1 diabetes

T2D: type 2 diabetes

VTA: ventral tegmental area

WTCCC: wellcome trust case control consortium

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Introduction

1.1 Pleiotropy

One of the interesting observations of George Mendel during his study of inheritance in pea plants (Pisum sativum) was about the color of various plant parts. Particularly he found complete relatedness among seed coat color with flower color as well as axillary pigmentation. Mendel noticed that always plants with colored seed coats have colored flowers and colored leaf axils and plants with colorless seed coats always have white flowers and no pigmentation on their axils.[1]

Today, we know that Mendel's observation of complete association of phenotypes was the result of pleiotropy or the phenomenon in which a single gene influences multiple phenotypic traits. Accordingly, a new variation that happens in the gene may have an influence on some or all traits simultaneously. The underlying mechanism for instance can be because of the product of the gene is used by different cell types, or has a signaling function on a variety of targets.[2]

In some cases the influence of the mutation in final outcomes is straightforward; for example, the liver enzyme phenylalanine hydroxylase converts amino acid phenylalanine to amino acid tyrosine which is precursor for neurotransmitters like dopamine and norepinephrine, the hormone thyroxine, and the pigment melanin; thus, mutation in this gene can affect multiple body systems.[3]

However pleiotropy is not always straightforward and there are cases that mutation in a gene is beneficial for one trait and detrimental for the other trait. For example, the product of the TP53 gene has the ability to interrupt cell proliferation. It helps prevent cancer by stopping cells with DNA damage from dividing, but this ability can be

deleterious when it suppresses the division of stem cells which enables the body to renew and replace deteriorating tissues. Another example is the sickle cell anemia in regions that malaria is endemic, a single-nucleotide substitution at the sixth amino acid position of the β hemoglobin chain replaces glutamic acid with valine this causes a drastic change of erythrocyte shape (sickle-cell anemia); however, this mutation in regions that malaria is endemic protects against invasion of the protozoan parasite (Plasmodium falciparum) that causes malaria.[4-6]

This type of pleiotropy is called antagonistic pleiotropy which happens when one gene controls for more than one trait where at least one of these traits is beneficial to the organism's fitness and at least one is detrimental. It can make a major constraint on natural selection and results in more staying power of the genes with antagonistic pleiotropy in the evolutionary context.[4; 6; 7]

Although evidences of pleiotropy have been primarily found through the studies of simple disorders with proven Mendelian inheritance, recent studies suggest that the logic of pleiotropic genetic polymorphisms also extends to human disorders that have complex and largely unknown genetic factors. Previous studies reported numerous examples of a gene associated with disorders that are quite distinct. For example after a comprehensive search of NIH Genetic Association Database, Liu et al[8] found genes like angiotensin I converting enzyme (ACE), tumor necrosis factor alpha (TNF), tumor protein 53 (TP53) and apolipoprotein E (APOE) associated to more than 50 diseases. These genetic sharings among disorders have brought the concept of human disease network which indicates diseases themselves form a network in which two disorders are connected if they share at least one gene. Evidences form genetic and epidemiological studies also showed that certain hereditary disorders often co-occur significantly more or significantly less frequently than what is expected by chance which suggest there is a genetic component that predisposes its carriers to multiple disorders, or that protects against some disorders while predisposing to others.[8-12]

Dudley et al[13] systematically investigated the pleiotropy by doing the first genome-wide study of genetic pleiotropy. Authors created single gene deletion strains, representing all 4700 nonessential genes in yeast and analyzed the growth of these strains under 21 different conditions. They found 551 mutant strains, exhibited growth defect in only one or two conditions and 216 strains showed growth defects in 3–14 conditions, their findings indicated a much higher degree of pleiotropy than what is expected by chance even among the most dissimilar conditions tested and point to the importance of pleiotropy in biological systems.

Rzhetzky et al[12] reviewed 1.5 million medical records involving 161 diseases represent a broad spectrum of disorders affecting diverse physiological systems and computed pairwise correlations of disease co-occurrences. They found that disease phenotypes form a highly connected network of strong pairwise correlations; moreover, they noted striking tendencies for certain diseases to co-occur in individuals. They pointed that unlike the familiar model of "unique malady–unique (disjoint with others) set of broken genes" which is applicable to most Mendelian disorders, most complex phenotypes are probably rooted in genetic variations that are significantly shared (in either a competitive or cooperative manner) by multiple disease phenotypes and they recommend the design of genetic linkage or association strategies that analyze multiple complex disorders jointly for the disorders appeared to be correlated.

Using the data from Online Mendelian Inheritance in Man (OMIM) database that represents and up-to-date repository of all known disease genes and the disorders, Goh et al[11] constructed a network of human diseases in which two disorders are connected to each other if they share at least one gene. Although they found clustering among the disease of the same class but the resulted network did not fall into many single nodes corresponding to specific disorders or grouped into small clusters of a few closely related disorders; instead, it form a large network of human diseases. Of 1,284 disorders in OMIM database, 867 had at least one link to other disorders, and 516 disorders form a giant component, suggesting that the genetic origins of most diseases, to some extent, are shared with other diseases.

In another study, Barrenas et al[14] investigated shared genetic architecture of complex diseases studied using Genome Wide Association studies data from GWAS Catalog containing SNPs published with p-values less than 10^{-5}. They found from the 54 studied complex disorders, 26 disorders are sharing at least one gene.

1.2 Genetic mapping

1.2.1 Family-based vs. case and control association studies

Association studies examine the co-occurrence of a genomic markers and a phenotype in order to assess the contribution of genomic variants to a phenotype in specific population. Different types of association-based designs are used for mapping the genomic determinates of complex disorders. The two most widely used strategies are case-control studies of unrelated subjects and family-based designs.[15]

Case-control association study of unrelated subjects is the most simple and commonly used approach. A case-control study compares two groups that are expected to differ in their prevalence of disease-susceptibility alleles. Sufficiently large study populations can be readily assembled without the need to enroll family members of the recruited participants; however, population stratification may lead to spurious association and hence bias the findings. Population stratification happens when there is a systematic difference in allele frequencies among subpopulations in the dataset possibly due to different ancestry. In this case, false positive association can occur because of the confounding effects of population stratification. A number of approaches have been proposed to detect and account for population stratification in population based studies; however, it may be difficult to remove this effect in all situations; furthermore, there can be obscure relatedness in the samples collected for case-control studies that can violate the initial statistical assumptions.[15-18]

Family-based association methods evaluate whether particular alleles are transmitted from parents down to affected offspring in a proportion that is different from what is expected from Mendelian transmissions, an excess sharing of specific allele among the affected subjects indicates that a disease-susceptibility locus for a trait of interest is linked and associated with the marker locus. Because both linkage and association are required to reject the null hypothesis; hence, family based association tests avoid false positives that arise when association is present but linkage is not as might happen in the presence of population stratification.[15; 16; 19]

Collecting a sample of unrelated cases and controls is easier compared to collecting family-based samples; moreover, to get the same power as a case-control study design, a larger number of family-based samples will have to be ascertained and genotyped. Nonetheless, although case control studies are more feasible, family-based designs are still more appropriate mainly because unlike population-based studies, family-based designs are robust against population substructure, and significant findings always imply both linkage and association; moreover, compared to case-control designs, families tend to be less heterogeneous regarding exposure to environmental factors that are related to the disease etiology.[15; 16]

1.2.2 Genome wide linkage and association analysis

There are two main strategies for mapping the genomic factors behind the complex traits which are linkage analysis and association mapping. Both methods allow comprehensive scan of the entire genome for disease genes in a hypothesis-independent manner without having knowledge of the biology of disorder; however, each method has benefits and drawbacks.[19; 20]

Linkage analysis tests for co-segregation of a genomic marker and disease phenotype within a family in order to determine if the marker and the disease gene are physically linked. It estimates the recombination fraction between a disease locus of

unknown location and genomic markers of known location and evaluates whether this recombination fraction is significantly different from 0.5 (The situation that the disease locus is not linked to the genetic markers). Therefore, linkage analysis unlike the association studies requires a genetic map; in addition, compared to genome wide association studies, much lower number of markers are required for genome wide linkage analysis; for instance, a typical genome wide linkage analysis can be performed with about 600 markers corresponding to marker-marker intervals of 5 centi Morgan (cM).[20; 21]

Linkage analysis has been the primary method for identifying genes underlying Mendelian disorders. Although there are examples of disease alleles of modest effect that have been detected by linkage analysis, this approach has been more successful in detecting genes with large effect; whereas, most of the genetic variance in complex disorders can be attributed to genes of modest effect which are more likely to be captured with association studies. Besides, association studies provide greater resolution of location than linkage studies since they do not rely on recombination rate.[19-22]

Overall, in many ways both linkage and association provide complementary data and using a joint approach allows combining the merits of both methods; for instance, performing association tests within region flagged by linkage signals will provide more robust results and also remarkably reduce the number of SNPs for multiple testing.[21]

1.2.3 Founder populations

Linkage and association analysis can be augmented by the study of founder population. A founder population is descended from a limited number of individuals (founders) as a consequence of some type of bottleneck.[23] Samples of individuals from founder populations have already proved greatly useful in mapping genes that underlie Mendelian disorders. It is becoming increasingly apparent that studies locating genes underlying complex phenotypes also benefit from the study of samples from founder population in several ways.[24-26]

In a founder population fewer numbers of haplotypes being segregated through the generations compared to outbred population. This reduces the number of markers for genome wide scans; moreover, in a more outbred population with considerably higher numbers of haplotypes, the causative allele is more likely to be located on several haplotypic backgrounds, thereby diluting signals to an extent that prevent its identification by genetic means; then, the value of population isolates and their genomic LD patterns may thus be even greater when lower-frequency variants are considered.[24-26]

Founder populations display higher degree of genetic homogeneity because of the limited number of founders (and thus a limited gene pool) and absence of migration, this diminish the risk of unidentified population stratification that can bias results and compromise the interpretation of genetic association studies; in addition, extensive allelic and locus heterogeneity, a key feature of common complex diseases that can obscure the association signal within disease-associated genomic regions is lower in founder population compared to outbred populations.[24-26]

Unlike the monogenic disorders where the genetic composition of an individual often solely determines the disease phenotype, environmental factors are critical risk factors in complex diseases; therefore, since environmental risk factors are generally more uniform in founder populations than in large outbred populations, probing the genetic determinants of complex disorders seemingly are easier in a founder population compared to outbred population. Furthermore, the availability of extensive genealogical records in a founder population can provide large genealogies which are potentially very informative in genetic studies.[24-27]

1.3 Saguenay-Lac-Saint-Jean population

The French colonization of Quebec began from 1608 until 1760 in which about 8000 to 10000 people who came from France, permanently established themselves in the province. This establishment has been referred to as the first founder effect.[28-30]

This founder effect was further augmented in some sub populations of Quebec including the Saguenay-Lac-St-Jean (SLSJ) region located about 200 km northeast of Quebec City. The initial settlement of SLSJ region occurred in the 19th century, all regions of the province of Quebec have contributed to the growth of SLSJ population, but the majority of migrants (about 75%) in the earliest decades came from Charlevoix, a region located on the north shore of the St. Lawrence River which is about 200 km south and east of Saguenay. The settling of the Charlevoix region itself started in 1675 in which 599 founders of mostly French descent moved to this region from the Quebec City area. There has been relatively little migration into the SLSJ region after 1870, and the population has intrinsic growth from 5200 in 1852 to about 300,000 at present.[29; 31-33]

As a consequence of the SLSJ population history, the prevalence of several recessive disorders is higher in the SLSJ region than in other populations such as myotonic dystrophy, spastic ataxia, tyrosinemia, agenesis of the corpus callosum and vitamin D-dependent rickets. [31; 34; 35] Labuda et al[31] using a demographic model suggested the historical age of the founder effect in SLSJ is about 12 generations; therefore, in this population with fewer number of haplotypes being segregated over the time, even longer stretches of LD and less heterogeneity are expected compared to older founder populations.

Founder effect also causes limited allelic diversity; for example, a single homozygous mutation was found in 80% of patients with hereditary tyrosinemia type I, and only three mutations were found in 94% of patients with cystic fibrosis pointing to the reduced genetic heterogeneity in this population. It is also reported that large blocks of ancestral DNA flank the disease mutations in this population; specifically, recent studies have demonstrated that ancestral haplotypes spanning about 8-10 cM are common to 40%-60% of affected chromosomes and blocks about 4-6 cM being common to nearly 80% of populations with rare diseases from this region.[31; 36-38]

Moreover, extensive genealogical records exist in the BALSAC population register maintained at the inter-university Institute for Population Research provides additional benefits for genetic studies.[163] The register contains ascending genealogies dating back to

the original 17th-century settlers; they have been constructed on the basis of parish records of baptisms, marriages, and burials of 1,500,000 individuals. To date, all records have been computerized, and families reconstituted into a genealogically linked database.[27; 39]

1.4 Substance use

Alcohol, tobacco and coffee are among the most commonly consumed psychoactive substances in the world. Their concurrent use has been consistently shown across a wide variety of populations. Previous studies have reported relatively strong correlations between smoking and alcohol consumption and smoking and coffee consumption. Coffee and alcohol consumption are also correlated especially when either substance is used heavily. [40-43] Abuse and dependence risks have been demonstrated for these substances and some researchers postulate that there is a relationship between the use of these substances and that of illicit drugs; moreover, in comparison with other substances, the high prevalence of alcohol, tobacco and coffee use allows for significant statistical power in a practically sized sample. Probing the relatedness of these legal psychoactive substances may also provide information for elucidating factors contributing to the use, abuse, and dependence of other drugs; moreover, such information can provide tips for prevention, intervention and treatment programs.[40-43]

Several models have been proposed to explain the correlations in consumption of these substances; for instance, biobehavioral models suggest that the use of one substance prompts the use of other types of substances, personality models point to presence of underlying psychological trait or set of traits that predispose an individual to polysubstance use and the neurogenetic models suggest, the commonalities in the use of substances are due to common neural pathways and receptors in which psychoactive substances act and interact.[40; 43; 44]

Genetic studies utilizing twins and family approaches have shown that substance use is substantially heritable;[46] in addition, animal studies also point to the importance of

genetic factors; for example, under similar environmental settings, the Lewis rats more readily self-administer drugs of abuse compared to Fischer 344 rats which indicate differences in genetic liability, additional studies showed that these strains differ in properties of their mesolimbic dopamine system.[45] Clinical and experimental studies also documented that genetic factors operate at all steps of substance use, including vulnerability to initiation, continued use, propensity to become dependent and relapse to drug taking or drug seeking after a period of abstinence. In this context, different genetic models have been proposed to account for the clustering in the use of these substances. One prevailing hypothesis indicates that there is a general non-specific genetic risk factor that increases subjects's liability to use multiple psychoactive substances for instance a common neural pathway.[44; 46]

In fact, although psychoactive substances have their specific receptors, mechanisms of action and pharmacological effects, all appear to influence brain's reward pathways by producing a series of common functional effects after both acute and chronic administration. Reward system includes dopaminergic neurons in ventral tegmental area (VTA) of the midbrain and their targets in nucleus accumbens (NAc) and prefrontal cortex. The VTA-NAc pathway is one of the most important substrates for the acute rewarding effects of all drug of abuse and research over the past several decades has documented how each drug, regardless of its distinct mechanism of action, coincide on the ventral tegmental area and nucleus accumbens with common acute functional effects. Enhancement of dopaminergic transmission in nucleus accumbens has been implicated in the mechanism of reinforcement of almost all psychoactive substances. All addictive drugs facilitate dopamine transmission, In fact determining the role of dopamine has been an important focus of biomedical research in addiction. Chronic drug states are also appear to induce similar changes in central corticotropin releasing factor (CRF) systems. Abrupt withdrawal from virtually any drug of abuse leads to activation of CRF-containing neurons in the amygdale. Activation of these neurons during drug withdrawal partly is responsible for the negative emotional symptoms as well as many of the somatic symptoms that occur upon drug withdrawal and therefore may contribute to drug craving and relapse.[44; 46; 47]

In this context, another model proposes that genetic risk factors are largely substance specific such as variation in receptor systems specific to individual drugs of abuse. For instance, nicotine acts through specific nicotinic receptors distributed throughout the central and peripheral nervous systems. Caffeine has similar structure to adenosine and its principal mode of action appears to be on adenosine receptors. Less is known about alcohol specific receptor systems in the brain, but it has been shown to affect gama-aminobutyric acid (GABA) ergic neurons and glutamatergic systems as well as serotonin and endorphin release.[40; 41; 43; 44]

Number of studies investigated the source of covariations among substance uses across populations. Kozlowski et al[42] looked at the relationships among the frequencies of use of various drugs in two drug abusing populations. They found that among their studied drugs, use of tobacco, alcohol, and caffeine are directly related to one another and they concluded that use of these substances may be governed by similar factors.

Swan et al[40] performed a multivariate genetic analysis of tobacco, alcohol, and coffee consumption in a cohort of 173 monozygotic and 183 dizygotic male twin pairs from Twin Registry of white male World War II veterans to determine the extent of genetic and environmental overlap in the observed correlations among these substances. Their best fitting model found a common genetic component which they called it polysubstance use factor underlying the observed correlations among alcohol, tobacco and coffee use. Residual genetic variances specific to the use of each substance were also identified; moreover, they found no significant role for common environmental variance in their hypothesized polysubstance use factor.

In another study performed by Hettema et al[41] in a sample of 774 monozygotic and 809 dizygotic male and female twin pairs from members of the population-based Virginia Twin Registry. Authors probed whether the overlap of use of tobacco, alcohol, and caffeine is due to non-specific shared genetic or environmental substance use factors or due to factors that are highly substance specific. Their findings suggest that the variance is

proportioned into both shared and specific genetic and environmental factors that are of moderate size.

In a more recent study, Li et al[48] conducted a meta-analysis on the basis of manually integrating 2343 items of cross-platform data linking genes and chromosome regions to addiction from peer-reviewed publications spanning 30 years from 1976 to 2006. Focusing on 396 genes that were supported by two or more independent items of evidence they found 18 statistically significant enriched pathways in which Long-term depression, Gap junction and Long-term potentiation were the most significant pathways. Focusing on alcohol, nicotine, cocaine and opioid they identified five common and interlinked pathways behind these substances including Long-term potentiation, MAPK (mitogen-activated protein kinase) signalling pathway and Neuroactive ligand–receptor interaction that had been previously implicated in addiction as well as two new pathways; GnRH (gonadotropin-releasing hormone) signalling pathway and Gap junctions.

Many studies that have investigated the importance of genes in use of psychoactive substances also suggest substantial contributions from the environment. Among various environmental factors that may influence substance use, stress seem to be an important factor, it has been reported that stress increases the vulnerability of individuals to acquisition of substance self-administration and it is considered as an important factor in addiction research.[46; 49; 50]

1.5 Substance use and stress

The term stress was first employed in a biological context by the endocrinologist, Hans Selye in the 1930s. He described the hypothalamic-pituitary-adrenal axis as the system whereby the body copes with stress and found the principal remaining problems and misconceptions surrounding the clinical application and theoretical evaluation of the stress concept.[51; 52]

Stress is a well-known risk factor and precursor to use of psychoactive substances. Several models of substance abuse consider acute or chronic stress as an important contributing factor in development of substance abuse such as stress-coping model of addiction, Marlatt's relapse prevention model, tension reduction hypothesis and self-medication hypotheses. These models mainly point that people use substances to reduce negative affections and enhance positive moods under acute and chronic stress conditions.[53]

Numerous studies in experimental and clinical research documented that stressors facilitate the acquisition of psychoactive substances, stressors can also increase the rate and dosage of substance use and stressors can cause relapse to, or reinstatement of drug taking even after a prolonged drug-free period.[46; 53; 54]

A growing body of evidences point to a number of biological connections between substance use and stress. Based on findings from clinical studies, one model postulates that stress leads to state-related changes in the brain reward circuits resulting in a greater sensitivity to the reinforcing properties of drugs, and thereby increasing the motivation to use drugs compulsively. In fact, chronic exposure to either stressors or substance abuse appears to exert many similar changes in the brain's reward pathway via dopaminergic and glutamatergic neurotransmitter systems. For example both stress and substance use increase the release of dopamine in nucleus accumbens, activation of dopaminergic pathway either by stressors or drugs exerts similar changes in growth factors, transcription factors and second messenger cascades in the midbrain ventral tegmental area and its terminal fields, following exposures to either stressors or substance abuse reorganization of synaptic connections of reward pathway has been observed; moreover, other studies have pointed to the importance of gluamergic system in the reward pathway, for instance, it was reported that immobilization stress, morphine and cocaine all induce the up-regulation of the GluR1 subunit of the AMPA receptor for glutamate in the VTA.[47; 55; 56]

While reward pathway appeared to be the central players in addiction, evidences like tendency to relapse years after a prolonged drug-free period indicate that brain regions that are involved in memory and learning may also have function in addiction. In fact, an

influential hypothesis points that addiction represents a pathological yet strong form of learning and memory. Previous studies documented that drug seeking can get inhibited by disrupting the reconsolidation of drug-related memories and stress on the other hand is known to influence different stages of memory and learning. For example, it has been shown in rats that immobilization stress facilitates morphine self-administration if it comes after the onset of the drug availability session indicating the importance of associative leanings.[44; 47; 56]

The brain structures that are involved in memory and learning, such as hippocampus and extended amygdala are connected with brain reward pathway and interestingly both stress and drug exposure impact the structure and function of these regions. In general, similar neuroplasticity or neuroadaptations seem to occur in these regions after exposure to stress or drugs.[56-58]

The recruitment of brain-stress systems within the extended amygdala provides a powerful mechanism for seeking and taking psychoactive substances and evidences suggest dysregulation of amygdale influence both stress response and substance use; for instance, it has been reported, rats with the damaged amygdala are less responsive to dangerous stimuli and are more sensitive to cocaine use compared to normal group. [59] Neurogenesis is very active in the hippocampus; similar to stress, chronic exposure to drugs of abuse also impact the neurogenesis and neural structure and function in hippocampus and similar to reward pathway, both stress and drugs of abuse appear to influence these brain structures via dopaminergic and glutamatergic neurotransmitter systems as well as via glucocorticoids released upon activation of HPA axis to alter neural structure and function.[56; 60]

A main component of body's response to stressors is the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1). HPA axis activation or suppression influences use of psychoactive substances. Hormones released upon activation of HPA axis like glucocorticoids can alter neural structure and function in reward pathway as well as learning and memory processes and basal levels of stress hormones seem to contribute to behavioral responses and neuroadaptations to drug exposure; for example, substances like

cocaine, morphine, nicotine, and alcohol are reported to be differentially reliant on stress hormones for their behavioral impact. Drug-induced activation of HPA axis allows glucocorticoids to sensitize the reward pathway. This sensitization of the reward system would make the subjects more responsive to drugs of abuse and, consequently, more vulnerable to the development of addiction.[46; 47; 56]

Substance use can change the activity of HPA axis; for instance, it has been found that patient with heroin dependence are having a hypo-responsive HPA system and those with dependence on cocaine show a hyper-responsive HPA axis;[46] in addition, Lewis inbred rats that more readily self-administer drugs of abuse compared to Fischer 344 rats are also having hypo-responsive HPA axis responses to stress exposure and it has been shown that exposure to stressors increases corticosterone and/or ACTH (adrenocorticotropic hormone) levels in F344 but not in Lewis rats.[46; 61]

Previous studies using opioid antagonists documented that the endogenous opioid system, via opioid receptors demonstrate inhibitory control over the HPA axis (Figure 1); moreover, mice lacking the mu opiate receptor gene (OPRM1) show dramatically reduced or absent analgesia, reward, physical dependence and respiratory depression in response to opiates. The most common coding region polymorphism in this gene is a variant (A118G) that changes asparagine (Asn) to aspartic acid (Asp) at amino acid position 40. In vitro studies showed that beta-endrophine binds to the new form, 118G (Asp40) with threefold greater affinity than the prototype 118A (Asn40) receptor; besides, clinical studies found that carriers of 118G allele showed a greater HPA response to opioid antagonist than subjects with the only prototype 118A allele. Additionally, people with the 118G allele had a more favorable clinical response to treatment for alcoholism with the opioid antagonists. This indicates that the difference in response to treatment may be mediated by the impact of the receptor on HPA axis activation; moreover, it has been reported that basal amounts of cortisol in subjects with the 118G allele are significantly higher than in volunteers with the only prototype form in a stress-minimized setting condition.[46; 62-64]

Another evidence of genic link among HPA axis, stress response and addiction was identified through the studies on COMT gene, which encodes an enzyme that catalyzes the degradative metabolism of catecholamine neurotransmitters dopamine, norepinephrine and epinephrine, as well as hydroxylated estrogens. A common variant in exon 4 of this gene change valine to methionine at amino acid 158. Methionine form has greater thermolability and a three- to fourfold lower enzymatic activity than the prototype valine form. Genetic linkage and association studies pointed that this variant may be involved in several centrally mediated traits. Several studies documented that the low-activity of methionine form is associated with increased risk of alcoholism; moreover, it has been repoted that this variant influences HPA axis function; for instance, after administration of naloxone, subjects with homozygous methionine form had higher increases in their plasma ACTH and cortisol than heterozygous or homozygous subjects for prototype valine form.[46; 65-67]

Endocannabinoid system that mediates the psychoactive effects of cannabis has also been elucidated as a critical regulator of the stress response through its ability to modulate the sensitivity and activation of the HPA axis (Figure 1). Under conditions of acute stress, the endocannabinoid system tonically restrains activation of the HPA axis and disruption of endocannabinoid signaling in the other hand increases the activity of the HPA axis and is associated with an inability to adapt to chronic stress.[164-166]

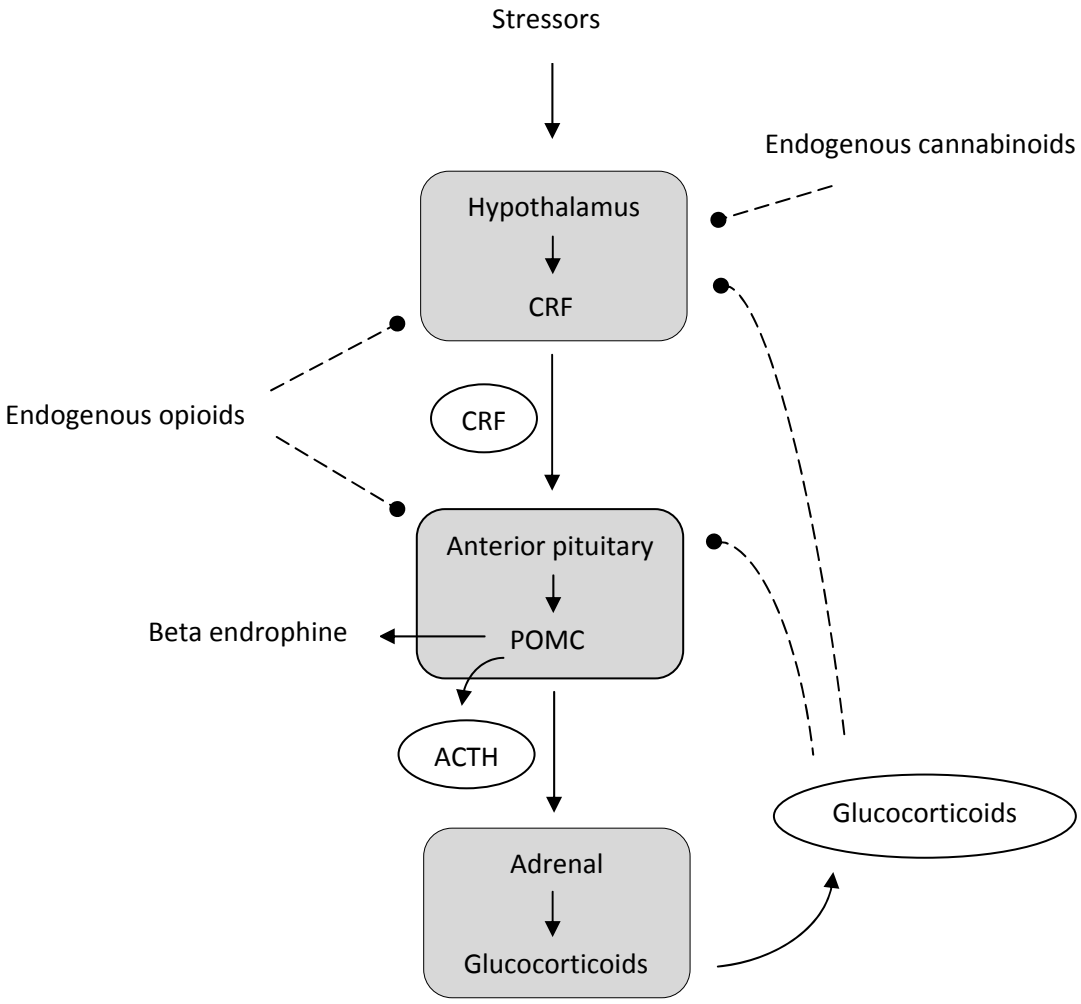

Figure 1. Stress-induced activation of the HPA axis.

Stressors cause increased synthesis and release of hypothalamic corticotropin releasing factor (CRF) into blood circulation. By binding to its specific receptor in the anterior pituitary, CRF induces synthesis of proopiomelanocortin (POMC) and release into the circulation of adrenocorticotropic hormone (ACTH) and beta-endorphin, which are derived from processing of POMC. ACTH acts on its specific receptor in the cortex of adrenal gland and induces release of the glucocorticoid hormones. Glucocrticoids exert negative feedback regulation at both the hypothalamus and the pituitary to decrease the activity of HPA axis. In addition to negative feedback regulation by glucocorticoids, the endogenous opioid and cannabinoids systems tonically constrain activation of the HPA axis.

1.6 Substance use and hypertension

Excessive alcohol intake increases the risk of many diseases and is reported to be the commonest cause of reversible hypertension. Reduction of heavy alcohol intake is considered in hypertension management programs; however, considerable research suggests that moderate alcohol consumption is associated with health benefits including a decreased risk of hypertension. It appears that the effect of alcohol use on hypertension is in a form of J-shaped correlation suggesting the benefits of moderate alcohol intake; however, the modulatory effect of alcohol on hypertension also depends on other factors including environmental factors as well as genetic factors. [68; 69; 71] For example, the level of sodium intake is positively correlated with increased blood pressure only at low calcium intake and this correlation was more significant in subjects who consume high amounts of alcohol.[70] Genetic factors also influence the mediatory effect of alcohol on hypertension; for instance, variations in alcohol-metabolizing enzymes such as CYP2E1, ADH2, and ALDH2 genes modify the intake of alcohol and as a result the relationship between alcohol intake and blood pressure.[68] Another interesting example of genetic link between hypertension and alcohol use comes from the study of apolipoprotein E gene (APOE). APOE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and hence is important in regulation of serum low-density lipoprotein (LDL) cholesterol concentration. The APOE gene accounts for approximately 7% of the population variance in total LDL cholesterol levels. This gene exerts its heightening impact on LDL cholesterol through the APOE4 allele which is associated with higher LDL cholesterol, while carriers of the APOE2 allele have lower LDL cholesterol levels.[68; 72]

Findings from the Framingham Offspring Study showed that the effects of alcohol intake on LDL cholesterol are mediated in part by variations in APOE gene. Corella et al[73] noted that in the group of male non-drinkers, LDL cholesterol was not significantly different across APOE allele groups; however, in the group of male drinker the expected difference was observed where subjects with APOE4 allele had higher LDL cholesterol compared to the APOE2 allele carriers; moreover, they found LDL cholesterol in men with

the APOE2 allele was significantly lower in drinkers than in nondrinkers but was significantly higher in drinkers than in non-drinkers in men with the APOE4 allele.

APOE alleles also appear to impact the blood pressure;[72] for instance, in a recent study, authors did a meta-analysis of six studies comprising 1812 cases and 1762 controls in an effort to systematically investigate the association between APOE gene variations and high blood pressure. They found that presence of APOE4 allele is associated with increased risk of developing hypertension.[74]

One study[75] found that in males that are moderate or heavy drinkers, the APOE2 allele may enhance the blood pressure-elevating effects of alcohol, whereas the APOE4 allele seems to abolish it. These interactions among alcohol intake, APOE allele type and blood pressure were not detected in females, suggesting a sex-specific effect; however, another study found no relationship between decrease in blood pressure with alcohol restriction and apolipoprotein APOE genotypes,[76] which points to the requirements for further studies.[68; 77]

Another example comes from the study of GNAS1 gene that encodes the alpha subunit of G-protein involved in cAMP-dependent pathway by activating adenylate cyclase. Chen et al[78] assessed the interaction between the polymorphism T393C in this gene and alcohol consumption in association with hypertension in a Japanese population consisted of 699 hypertensives and 1609 normotensives. Their results suggest that the apparent effect of the T393C polymorphism on blood pressure depends on alcohol consumption. They found that the T393C polymorphism significantly interacted with drinking status in association with systolic blood pressure; moreover, while subjects with the TT or TC genotype consistently had a higher probability of hypertension, higher systolic blood pressure, and higher diastolic blood pressure than CC homozygotes in non-drinkers and light drinkers. This relation was invert in the group of moderate to heavy drinker where subjects with the CC genotype consistently had a higher probability of hypertension and higher SBP and DBP than subjects with the TT and TC genotypes.

While there are a substantial number of studies that documented the adverse effects of smoking on health, the role of smoking as a risk factor for hypertension is not established. Tobacco smoking temporarily raises blood pressure (BP) probably through vasoconstriction and accelerated heart rate; nonetheless, results from epidemiologic studies have generally shown that smokers have lower BP compare to nonsmokers; meanwhile, former smokers have BP similar to those of nonsmokers. Gene-environment interaction findings pointed that genetic factors are important mediator of the effect of tobacco smoking on hypertension.[79; 80; 167]

In the same cohort in which authors found a significant interaction between the T393C polymorphism in GNAS1 gene and alcohol use in the pathogenesis of hypertension [78], they[81] also observed that the TT and TC genotypes of the T393C polymorphism have a risk-increasing effect on development of hypertension in non heavy smokers and heavy smoking significantly decreased the effect. In contrast, the CC genotype had a relatively protective effect on the development of hypertension in non-heavy smokers, and heavy smoking did not modify the effect significantly. Authors concluded that since subjects with the TT and TC genotypes are more common compared to those with the CC genotype; hence, their findings are consistent with the evidences that smokers have lower BP compared to nonsmokers. They also pointed that the observed protective effect of smoking is not attributable to the phenomenon that smokers have lower body mass index than nonsmokers because the T393C polymorphism appears to have no significant interaction with body mass index in association with hypertension.

Another line of evidence of genes and smoking interaction in the development of high blood pressure came from studies of ACE gene which encodes angiotensin I converting enzyme (peptidyl-dipeptidase A) that is involved in renin-angiotensin system and catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II. A variation in this gene result in D allele which is associated with increased ACE activity compared to I allele. In a cohort of 412 non-smokers, 2794 former smokers and 1508 current smokers, Schut et al[82] assessed the relationship between the ACE I/D

polymorphism, systolic (SBP) and diastolic (DBP) blood pressures and risk of hypertension in each group. They found a significant association between the ACE I/D polymorphism, SBP and the risk of hypertension in smokers. Individuals who smoke and carry the D allele showed significantly increased SBP and an increased risk of hypertension compared with those who smoked and had the II genotype; meanwhile, they found no relation between the ACE genotype and blood pressure or risk of hypertension in non-smokers and former smokers groups.

Caffeinated beverages are widely used in societies. It is not clear whether there is a causal relationship between caffeine and hypertension. Findings from studies that investigated the effect of coffee intake and hypertension are inconsistent while some showed no effect, other studies found positive relation or inverse relation. It has been suggested that these discrepancies in results may be in part due to differences in genetic risk factors.[83; 84]

Cytochrome P450 1A2 or CYP1A2 is the main enzyme responsible for the metabolism of caffeine. A variation in this gene results in allele A and F. Subjects homozygote for allele A are fast caffeine metaboizers compared to those with FF and AF genotypes. Palatini et al[84] investigated the effect of coffee intake on the risk of developing hypertension among individuals screened for stage 1 hypertension. They noted that after 8.2 years of follow-up the heavy coffee drinkers homozygote for allele A have 9 mmHg lower BP than their counterparts with FF and AF genotypes.[84] Consistent with this finding, another study suggested that coffee consumption increases the risk of myocardial infarction only among individuals with slow caffeine metabolism genotypes.[85] These findings point to the importance of genetic factors in studying the relation of coffee intake with hypertension.

1.7 Substance use and obesity

In 1949 Donald Hebb proposed that starvation is a learned behavior, in which eating is initially reinforcing because it revokes unpleasant body signals e.g. change in nutrient levels and hunger hormones in the blood and over the time it finally becomes an organized behavior. Findings from studies that investigated the biological connections among substance use and feeding behavior also appear to accumulate at the neurobiological level and suggest the importance of findings from the field of drug addiction in obesity research.[86-89]

Opioid receptor subtypes that as mentioned earlier provide genic link among substance use and stress also influence the regulation of food intake. The opiate antagonist, naloxone inhibits feeding in mammals, slugs, snails and even in amoebae; while, opioid contributes to increased food intake by delaying satiety signals during a meal.[87; 90; 91]

Rats that are fed palatable diet are more sensitive to the anorectic effects of the opioid receptor antagonists. This indicates that palatable foods are also able to change the activity of endogenous opiate system. Findings from experimental studies also documented that the opiate receptors and their ligands are dysregulated in several animal models of obesity for example mu receptors are up regulated in diet-induced obese rats and concentrations of endogenous opioid peptides were elevated in obese mice having the mutated form of leptin gene, consistently clinical studies showed that obese subjects have higher level of beta-endorphin compared to normal subjects.[44; 92]

Among various opioid receptor subtypes that play a role in regulating energy balance, the mu opioid receptors appear to have more important function. Mu receptors are mostly located in brain areas controlling feeding and rewarding behavior. Modifications of mu opiate receptors have been reported in the hypothalamus of rats susceptible to obesity induced by eating a high fat diet. Recent studies on rodents indicate that the stimulation of mu opioid receptors preferentially increases the intake of highly palatable foods whereas their block exerts anorectic effects; moreover, there are evidences that administration of opioid agonist, morphine into the nucleus accumbens of rats increases food intake. These

findings suggest opioid receptors control the appetite through the hedonic processes associated with reward pathway.[44; 91; 92]

Similar to opioid system, endocannabinoid system has also been reported to be directly involved in feeding regulation, infact the ability of cannabis to promote eating has been known for many centuries. Interstingly, now there are convincing evidences that link both endocannabinoid system and opioid system in the reciprocal modulation of hedonic factors that mediate feeding behavior.[168-170] It has been shown that stimulation of cannabinoid type 1 receptor by cannabinoids heightened intensity of food craving and enhanced appreciation of food;[168-170] this subsequently led to development of specific cannabinoid type 1 receptor antagonists such as Rimonabant as anti-obesity medication.[171]

Both feeding and drug use involve learned habits and preferences that are stamped in by the reinforcing properties of powerful and repetitive rewards. Pharmacological blockade of, or experimental damage to forebrain dopamine systems attenuates free feeding and lever-pressing for food reward, as well as the rewarding effects of psychoactive substances. Neuroimaging scans suggest reduced brain activity of dopamine may contribute to obesity as well as drug addiction.[44; 92; 93]

In fact, highly palatable food appeared to have properties that promote dependence; moreover, similar to drugs of abuse, palatable food can activate the brain reward system. Dopamine reuptake inhibitor, methylphenidate increases brain synaptic dopamine, produces anorexia, and finally reduces the intake of highly palatable food. In animal models of obesity including obese Zucker rats, leptin-deficient ob/ob mice and seasonally obese animals treatment with dopamine receptors D2 and D1 agonists reverses the obesity; however, connection between dopamine receptors and food intake does not appear to be straightforward. Dopamine acts through several dopamine receptors (D1–D5) which seem to mediate distinct effects on food intake and food preference. It has been noted that selective activation of Dopamine receptor D1 (DRD1) resulted in increased caloric intake and preference for highly palatable foods, whereas combined activation of DRD2 and DRD3 receptors showed an opposite effect. The reduced activity of dopamine neurons and

dopamine D2 receptor availability in the ventral striatum are also reported to be associated with both obesity and drug addiction. Repeated stimulation of the reward pathways through highly palatable food may lead to neurobiological adaptations similar to substance abuse that eventually increase the compulsive nature of overeating characterized by frequent drive to initiate eating.[87; 93-96]

In comparison to drugs of abuse that activates the reward pathway in a rather direct pharmacological way, palatable food appeared to act through both, fast sensory inputs as well as slower post ingestive processes such as adiposity signals, leptin and insulin that have important function in energy regulations; however, leptin and insulin are thought to decrease food intake partly by modifying the reward value of food, for instance, intraventricular administered leptin and insulin are able to decrease sucrose self-administration, additional support of the influence of satiety signals on reward pathway came from the observation of insulin and leptin receptors on the ventral tegmental area which is a key structure of the brain reward circuitry.[97-99]

Studies in rats documented that removing the endogenous corticosterone source, largely or completely eliminate obesity. Administration of glucocorticoids has been shown to markedly increase food intake. HPA axis can modulate the influence of leptin and insulin on food intake. Increased glucocorticoid concentrations have been associated with insulin resistance as well as leptin resistance; thus, elevated levels of cortisol could lead to impaired sensitization of satiety signals and inadequate adjustment for excess weight gain of the organism. Glucocorticoids like cortisol can influence drug use and food intake also through reward pathway. In basal conditions, administration of glucocorticoids increase dopamine release in the nucleus accumbens, whereas suppression of glucocorticoid secretion has the opposite effects; moreover, the opioid receptors that play important role in food intake through the reward pathway, are having connections with HPA axis. Variations in mu opioid receptor influence the level of glucocorticoids; besides, it has been shown that the expression of the mu opioid receptors and hence, opioid sensitivity is modulated by glucocorticoids for instance the expression of the mu opioid receptors are diminished in

CRH-deficient mice but it can be reversed by corticosterone administration. Endogenous opioids are a group of the neurotransmitters that are released upon activation of HPA axis. They act as part of body's powerful defense mechanism against the detrimental effects of stress. Opioids decrease the activity of the HPA axis on different levels in order to terminate and attenuate the stress response, providing a negative feedback control mechanism. As mentioned earlier, opioid release increases palatable food intake and palatable food sustains opioid release. Thus, food intake resembles a powerful tool to decrease HPA axis activation. If HPA axis activation becomes chronic and eating is learned to be effective, hence highly palatable food may have the properties similar to psychoactive substances.[87; 92; 93]

Genome wide scans also provide evidences of genic link between obesity and substance use. Findings from these studies suggest that obesity is a centrally mediated trait and share common neurobiological basis with substance use.[86; 100; 101]

A recent genome wide association study performed on more than 30,000 subjects participating in 8 large cohort studies found NRXN3 gene associated to obesity. [101] NRXN3 is part of a family of central nervous adhesion molecules and is highly expressed in the central nervous system; it is thought to be involved in synaptic plasticity. Prior studies of NRNX3 point to the important role of this gene in alcohol dependence, cocaine addiction, and illegal substance abuse. One study found that short-term cocaine exposure in mice is sufficient to increase the expression of NRXN3 in the globus pallidus; many of the neuronal pathways in these sub-cortical regions of the brain are involved in learning and reward training.[101; 102]

Findings on the two other well-replicated obesity genes, MC4R and FTO[103-106; 187] also suggest that obesity and addiction may share common neurologic underpinnings. Both of these genes are coding brain proteins. Variations in FTO gene appeared to impact food intake and selection and MC4R has been found to be associated with centrally-mediated phenomena including binge eating behavior. The FTO gene is almost expressed throughout the body, but is mainly abundant in feeding-related areas in the hypothalamus like the

arcuate nucleus. The arcuate nucleus has a direct projection to the lateral hypothalamic area which is implicated in reward and is one of the sites where electrical brain stimulation is most rewarding. The MC4R gene is also abundantly expressed in the arcuate nucleus as well as lateral hypothalamic area. Neurons of lateral hypothalamic area contain the neurotransmitter orexin which has been implicated in drug addiction. [86; 103-107] Another example of genic link between substance use and obesity comes from the study of dopamine receptor D2 (DRD2) gene. Individuals carrying the A1 allele of the Taq1A polymorphism that is located around 10 kb downstream of DRD2 gene have reduced brain DRD2 receptor density compared to those having the prototype form; in addition, this allele has been found to be correlated with obesity and persistent substance abuse.[86; 87]

1.8 Stress and hypertension

Regular exposure to stress as it happens in daily life may favor the development of chronic blood pressure elevation. It has been shown that exaggerated cardiovascular response to stress at young age may be predictive of the future development of hypertension. Findings from twin studies indicate that genetic factors account for 40% to 60% of the variance in cardiovascular reactivity to stress. It is postulated that repeated exposure to stress in combination with genetic susceptibility and unfavorable environmental factors may eventually lead to manifestation of hypertension. Various systems are reported to be behind stress induced hypertension and numerous genic variations have been found that links these systems to stress induced hypertension.[52; 108-114]

The sympathetic nervous system (SNS) which is part of the autonomic nervous system has an important role in mobilizing the body's resources under stress. Repeated exposure to environmental stress increases the activity of SNS and excessive activity of SNS in the long term appears to be positively associated with hypertension; for instance, peripheral vasoconstriction and increased renal tubular sodium reabsorption which are the consequences of long-term sympathoactivation, raise blood pressure too.[115-117]

Activation of SNS leads to the release of catecholamines, norepinephrine (NE) and epinephrine (EP) interestingly number of genes that are involved in catecholamine metabolism and transport are implicated in stress-induced-hypertension; moreover, some studies point to adrenergic receptors as a genic link between stress and hypertension which can regulate the cardiovascular response to SNS activation. Adrenergic receptor subtypes which are receptors for NE and EP influence BP by mediating heart function, renal sodium excretion, and vascular tone, another important group of genes that have been implicated in stress induced hypertension are dopamine receptors. Dopaminergic activity is involved in BP control as a negative modulator of SNS outflow, and there are numerous indications that hypertension develops in the absence of a normally functioning dopaminergic system for instance DRD5 knockout mice develop hypertension which is also characterized by increased SNS activity.[109; 118; 119]

One system that is activated as a result of SNS arousal is renin–angiotensin–aldosterone system (RAAS), an important regulator of blood pressure, over expression of renin and its metabolic products increase the blood pressure. The enzymatic cascade of this system includes angiotensinogen which is acted upon by renin to produce angiotensin I, which is in turn cleaved by a variety of enzymes, including angiotensin converting enzyme (ACE) to generate angiotensin II. This enzyme is a key player for most of the known biological activity of this system. Angiotensin II increases blood pressure via direct vasoconstriction. It also increases aldosterone production, and their combined effects eventually increase sodium and water retention that finally result in a rise in cardiac output and delayed BP recovery; besides, the augmented sodium retention enhances vasoconstrictive effects of norepinephrine on peripheral vasculature that further increases BP. It has been documented that increased angiotensin II production is associated with decreased urinary sodium excretion during a behavioral stress period which indicates the involvement of this enzyme in controlling stress-induced sodium retention; moreover, angiotensin II activates SNS and enhances the effect of NE; for example, infusion of angiotensin II increases muscular sympathetic nervous activity and angiotensin converting enzyme inhibition appeared to decreases muscular sympathetic nervous activity in

normotensive subjects. Other example of candidate genes in renin–angiotensin–aldosterone system that are implicated in stress induced hypertension are angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE), the Ang II type 1 receptor (AGTR1), and aldosterone synthase (CYP11B2).[109; 120-122]

HPA axis is a main component of body's response to stressors. Activation of HPA axis leads to the release of cortisol via a number of intermediate steps. First, the hypothalamus, at the base of brain triggers stress responses by producing corticotrophin releasing factor (CRF). Next CRF stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) which promotes cortisol secretion in the cortex of adrenal glands. Cortisol is thus a commonly used measure of HPA axis activation, and its secretion increases in response to any physical or psychological stress. As such, cortisol is often referred to as a stress hormone. Cortisol is related to hypertension; for instance, cortisol affects parts of the limbic system involved in the control of blood pressure regulation; furthermore, glucocorticoid receptors are also present in the kidney, heart as well as vascular smooth muscle of the resistance vessels, thus directly mediating the effect of cortisol on blood pressure. A number of monogenic forms of hypertension include pathophysiological changes in HPA axis function particularly cortisol biosynthesis and function have also been reported which again point to the modulatory effect of HPA axis on blood pressure.[109; 114; 123-125]

Another link between stress and hypertension comes from the study of heat shock proteins (HSP), HSP proteins are involved in cellular response to stress, they are a class of functionally related proteins whose their expressions are increased under the transcriptional control of heat shock transcription factors when cells are exposed to various stressors.[52; 112; 172] The increased expression of HSPs in response to several stressors has been previously reported by our group and other studies. It has been shown that in hypertensives, mRNA levels of these HSPs rose more rapidly but were followed by a more rapid decline to baseline for both mRNA and proteins as compared to normotensive subjects;[173-177] moreover, subjects with established hypertension have increased levels of HSP

antibodies;[178] besides, genomic loci containing HSPs have been found to cosegregate with cardiovascular traits.[111; 179]

In another study by our group, Thifult et al[113] used genome-wide linkage analysis to identify loci bearing stress-related phenotypes in recombinant congenic strains of mice. In A/J mouse strain that exhibits fear memory impairments with deficits in amygdalar long-term potentiation,[180] they identify stress QTL on mouse Chr 1 which was synthenic to a cluster of metabolic phenotypes of hypertension in human[27] and hypertensive dyslipidemic rats.[158] Following gene expression analysis, they found Atp1a2 gene in this region which was down regulated in the heart and brain of anxious mice compared to controls. Interestingly, this gene is also involved in the salt-related hypertension and exerting a major action on cardiac output and peripheral resistance,[181-183] which further support the pleiotropic function of this gene in stress response and hypertension.

Other important mediators that are implicated in stress-induced-hypertension are sodium reabsorption, endothelial system, serotonergic system and sympathetic nervous system; besides, growing body of literature have found number of genes involved in these systems that link stress to hypertension such as sodium channels in sodium reabsorption system, endotheline receptors in endothelial system, serotonin transporters and receptors in serotonergic system and cholinergic receptors in parasymphatic system.[109; 167]

1.9 Stress and obesity

Stress is known to be related to body weight. Some people lose weight and others gain weight in response to stress. It has been suggested that during stressful periods eating comfort foods or those high in fat and carbohydrate caloric content helps to reduce biological stress system activity and negative affections; in addition, lack of physical activity and time to prepare healthy meals during stressful periods further contribute to weight gain; however, chronic stress may also lead to weight loss particularly in subjects

whom chronic stress is associated with suppressed appetite and increased physical activity.[126-128]

Evidences suggest that the HPA axis, a central component of stress response and a common interface between substance use and cardiovascular disease is also involved in pathogenesis of human obesity, in particular characterized by visceral fat distribution.[92; 93]

As mentioned in the previous sections, variations in OPRM1 gene encoding mu opioid receptor were found associated to obesity and stress response. In fact, opioid provides a negative feedback control mechanism by reducing the activity of the HPA axis on different levels in order to terminate and attenuate the stress response. Variant 118G in exon 1 of the mu opioid receptor gene has a threefold increase in beta-endorphin binding. Bart et al[129] found that basal amounts of cortisol in subjects with the 118G allele in OPRM1 gene were significantly higher than in subjects with the prototype 118A allele in a stress-minimized setting, these findings indicated, mu opioid receptor modulates the HPA axis; moreover, it has been shown that the expression of the mu opioid receptor and hence, opioid sensitivity is modulated by glucocorticoids for instance its expression is diminished in CRH-deficient mice but it can be reversed by corticosterone administration.[62; 129; 130]

The endocannabinoid system has also been elucidated as a critical regulator of the stress response through its ability to modulate the sensitivity and activation of the HPA axis. Disruption of endocannabinoid signaling increases the activity of the HPA axis, which could involve a loss of inhibitory regulation of excitatory neurotransmission inside the neural stress circuit.[164-166] Interestingly, endocannabinoid system is also directly involved in feeding regulation. Stimulation of cannabinoid type 1 receptor, for instance by cannabinoids in addition to neuropsychiatric effects can also promote strong cravings for, and an intensification of the sensory and hedonic properties of food.[168-170] this led to the findings that the blockage of the cannabinoid type 1 receptor represented a novel pharmacological target for body weight reduction and later the discovery and development of cannabinoid receptor 1 antagonists.[171]

Glucocorticoids like cortisol or corticosterone that are released into blood stream as a result of HPA axis stimulation, mobilize energy resources from adipose and hepatic cells, ensuring a supply of energy. Dysregulation of glucocorticoids is related to obesity, for example the activity of cortisol on glucocorticoid receptors in both the hypothalamus and pituitary which inhibit HPA axis activity seemed to be impaired in visceral obese subjects; furthermore, hypercortisolism is correlated with symptoms including weight gain while hypocortisolism induce body weight loss. Genetically modified mice that have very low levels of glucocorticoids, will not develop obesity even if they maintained on a high-fat diet. It has been shown that glucocorticoids promote differentiation of pre-adipocytes into mature adipocytes and induce adipose tissue growth.[131-134]

Several polymorphisms in the cascade of the HPA axis have been found which cause differences in HPA functioning and also involved in obesity development. A variation in the exon 2 of POMC gene has been found to cause ACTH insufficiency and also associated with early-onset obesity. At the level of glucocorticoid action, a variation in intron 1 of the corticosteroid-binding globulin (CBG) has been associated to increased proliferation/differentiation of pre-adipocytes, higher salivary cortisol after dexamethasone suppression test, higher waist-to-hip ratio and a higher risk of obesity development; moreover, variations in the glucocorticoid receptor gene like N363S, NR3C1 and BclI have been found associated to obesity;[92; 135] moreover, the melanocortin subtype 4 receptor (MC4R); a well replicated obesity gene[184; 185] which is abundantly expressed in the arcuate nucleus and lateral hypothalamic area[86] has been implicated in stress-related behaviors as well.[186]

Connections among HPA axis and other pathway also appear to modulate the food intake. It has been shown that unlike glucocorticoids, CRH has anortic effect; for instance, obese fa/fa zucker rats are having a reduced level of CRH mRNA expression; moreover, intraventricular administration of CRH inhibits food intake in rats. This effect may be through pathways involving neuropeptide Y (NPY). Other studies found that while

glucocorticoids potentiate the orexigenic actions of NPY, CRH exerts inhibitory control on NPY-induced food intake.[136; 137]

Additionally, Central dexamethasone (a glucocorticoid receptor agonist) infusion stimulate NPY release in the mediobasal hypothalamus of female rats and NPY production of cultured hypothalamic neurons, recent studies reported that cortisol up regulates the NPY receptors in the abdominal fat by releasing the NPY; in addition, release of NPY and activation of the NPY receptors stimulates fat angiogenesis, proliferation and differentiation of new adipocytes, thereby linking HPA axis activation, NPY and increased abdominal fat storage. NPY appears to decrease anxiety and play an important role in the response to stress; therefore, it is also an important mediator of eating in response to stress.[138-140]

CRH is also suggested to be an important intermediate in the anorectic effects of leptin; moreover, rats which bear a mutation in leptin receptor gene have chronic increase of HPA axis activity, high levels of corticosterone and prominent visceral obesity. It has been shown that glucocorticoids stimulate the release of leptin by adipocytes; moreover, glucocorticoids are able to induce leptin resistance and thus attenuate the efficacy of leptin to suppress food intake, for instance, it has been shown that anorectic potency of leptin enhances in rats undergone adrenalectomy and this enhancement is reversible by glucocorticoid replacement. Induction of leptin resistance may also explain why glucocorticoid administration in humans results in elevated leptin concentrations and also an increased food intake.[141-143]

Glucocorticoids also influence release and action of insulin. Glucocorticoids interfere with insulin-induced glucose uptake and metabolism in both cultured myocytes and adipocytes in vitro. Dexamethasone administration decreases whole body insulin sensitivity in vivo. This is associated with a compensatory increase in plasma insulin concentration suggesting insulin resistance.[92; 93]

Leptin and Insulin are important adiposity and satiety signals that are released into the blood stream in proportion to adipose tissue. If obesity happens and adipose tissue

grows, increased satiety signals lead to reduced food intake so that excess weight will be lost. As mentioned earlier, increased glucocorticoid levels causes insulin resistance and leptin resistance; in addition, leptin and insulin are able to influence food intake partly by modifying the reward value of food. Insulin and leptin receptors are observed on the ventral tegmental area and administration of these hormones seemed to decrease sucrose self-administration. Therefore, elevated stress induced cortisol for a long period will impair sensitization of satiety signals and eventually result in inadequate adjustment for excess weight gain.[87; 97-99]

The stimulatory effect of glucocorticoids on energy intake appears to be through non-homeostatic pathways too. It has been shown that high cortisol levels leads to altered food preference and stress is thought to result in food choice for items with a higher content of fat and sweet which are perceived as highly rewarding, this suggests that stress influences food choice through reward pathway. In fact, several studies found cross links among dopamine reward systems and cortisol, or corticotropin releasing factor. Reward pathway a common interface between drug abuse and obesity appeared also to mediate the stress induced obesity. Cortisol can increase the sensitization of the reward system and sensitization of the reward system just like the case of substance abuse can lead to excessive intake of highly palatable food; moreover, similar to psychoactive substances, palatable food can activate the brain reward system, comprising opioid, dopamine and endocannabinoid signaling in the limbic system.[47; 92; 93]

Similar to drugs, palatable foods also appear to modify response to stress, for instance palatable foods facilitate release of endogenous opioids and dopamine in the limbic system. Palatable foods can activate opioid receptors in the ventral tegmental area and thereby stimulate cells that release dopamine in the nucleus accumbens and similar to psychoactive substances which can create dependency via this system; it has been suggested that palatable foods might also create dependency.[87; 96]

1.10 Obesity and hypertension

Obesity and hypertension are important public health challenges because of their high frequency and concomitant risk of cardiovascular, metabolic and kidney diseases. Both conditions have reached a pandemic proportion, imposing substantial costs on societies. Obesity is an important risk factor for development of hypertension. Findings from Framingham Heart Study showed that about 75% and 65% of hypertension cases in men and women, respectively, are attributed to obesity. It is also reported that a 5% weight gain increases hypertension risk by 20%-30% while weight loss reduces both systolic and diastolic blood pressures. Other studies have demonstrated a positive linear correlation between blood pressure and BMI in both normotensive and hypertensive subjects and even in subjects that were within the normal BMI range. The trial of antihypertensive Intervention and Management reported that a 4.5 kg or greater weight loss (about 5% of the baseline weight) reduce diastolic blood pressure to the same extent as a single-dose antihypertensive treatment. It is documented that not only the degree but also the distribution of accumulated body fat is an important risk factor for the development of hypertension; for instance, the prevalence of hypertension appears to be higher in individuals with upper-body obesity compared to those with lower-body obesity.[116; 144-147] Previous findings from our group also found that hypertensive and normotensive siblings drawn from the same families differ significantly by both degree and distribution of body fat accumulation and that genetic factors that co-segregate with hypertension appear to play a significant role in this difference;[148] moreover, the degree of genetic homogeneity increases in hypertensive-obese families compared to set of families selected at random or selected for hypertension and this improves the power to identify genetic causes of hypertension.[144; 149] In another study, following genomic analysis in two independent samples, Pausova et al[187] found that FTO gene, a well-replicated obesity locus, not only is related to obesity and insulin resistance, but also associated to hypertension which further emphasizes genetic relatedness of obesity and hypertension.

Both clinical and experimental data suggest that expansion of blood volume and increasing of renal sodium reabsorption are the central features in the development of obesity-induced-hypertension. The mechanisms by which obesity contributes to increased sodium reabsorption and blood volume expansion are not fully understood; however, increased sympathetic nervous system activity and activation of the renin–angiotensin system appear to play important roles. In fact, Pharmacological blockage of sympathetic activity through the adrenergic receptors attenuates hypertension more in obese subjects compared to lean hypertensive subjects; besides, other studies demonstrated that blocking of the renin–angiotensin system and of the aldosterone receptors also effectively attenuate blood pressure levels.[116; 145; 150]

Overweight is associated with increased sympathetic activity. Several observations suggest that over activity of the sympathetic nervous system is a major feature in causing obesity–hypertension in humans and animal models. For example, peripheral vasoconstriction and increased renal tubular sodium reabsorption that raise blood pressure are suggested to be the consequences of long-term sympathoactivation. Obese individuals are having higher muscle sympathetic neural activity compared to lean individuals; in addition, it has been shown that in obese individuals, the activity of sympathic nervous system is elevated in kidney which is a central organ in modulating the homeostasis of cardiovascular system. The effect of sympathic nervous system on obesity induced hypertension is also mediated through the cross links between the sympathic nervous system and other factors for example SNS stimulation can result in to renin–angiotensin–aldosterone system activation.[151; 152]

There are numerous evidences that point to the importance of the renin–angiotensin–aldosterone system in obesity-associated-hypertension. In obese subjects, increased circulating angiotensinogen, renin and angiotensin-converting enzyme activity have been reported; moreover, plasma renin activity declines with weight loss and this is also correlated with reduction in BP; besides, angiotensin converting enzyme inhibition is considered as an effective pharmacological means of lowering BP in obese hypertensive

humans. Adipose tissue expresses many components of renin–angiotensin–aldosterone system this suggests that high circulating angiotensinogen levels may be partially attributed to increased fat mass. A significant role of angiotensin II in stimulating renal sodium reabsorption and in contributing to obesity–hypertension is supported by the findings that treatment of obese dogs and obese hypertensive subjects with an angiotensin-converting enzyme inhibitor attenuates sodium retention and decreases blood pressure. The increased activation of the renin–angiotensin system appear to enhances the sympathetic activity, for instance, pharmalogical findings showed that blockage of angiotensin II receptor and angiotensin converting enzyme inhibition reduce muscle sympathetic neural activity; moreover, infusion of angiotensin II increases muscle sympathetic neural activity.[116; 145; 150]

Leptin is another mediator of obesity-induced-hypertension. Leptin is a 167 amino acid hormone which is expressed and secreted by adipocytes in proportion to fat mass. The effects of this peptide are mediated by receptors (Ob-R) which most of them located in the hypothalamus. Leptin decreases appetite and increases energy spending mainly by increasing the activity of sympathic nervous system. It appears that most obese persons are insensitive to endogenous leptin production; moreover, in obese people an interaction between high leptin levels and increased renal sympathetic tone has been observed. In fact the effect of leptin on blood pressure elevation can be prevented by blockage of sympathetic activity through the adrenergic receptors; in addition, it has been shown that infusion of leptin increases the sympathetic outflow to adipose tissue, kidneys, skeletal muscle vasculature and the neural traffic to the adrenal.[116; 153-155]

On the other hand, hormones like glucocorticoids or insulin can influence the level of serum leptin concentration. In fact, cortisol along with insulin and leptin creating a finely balanced system in order to provide sufficient fuel for the organism proportionate to needs.[92; 156]

In rats, marked leptin sensitivity was gradually diminished with glucocorticoid replacement and larger doses of glucocorticoids leads to overeating and consequently to obesity. Glucocorticoids stimulate secretion of leptin by adiposities; however,

glucocorticoids also reduce the efficacy of leptin to suppress food intake and therefore induce leptin resistance. The elevated level of insulin can affect leptin levels and increase the leptin concentration after meal.[92; 93; 131]

A growing number of studies suggest chronic stimulation of HPA axis and resulting excess glucocorticoid exposure may play a potential role in the development of obesity. Several DNA polymorphisms related to HPA axis functioning are also associated to the development of obesity and hypertension for instance hypercortisolism as it is observed in Cushing's syndrome causes symptoms like hypertension, insulin resistance, leptin resistance (as mentioned above) hyperglycemia and rapid weight gain. The increased cortisol secretion in primary obesity has been reported; besides, fatty tissue expresses enzyme, 11 Beta-hydroxysteroid dehydrogenase type 1 that convert cortisone to cortisol which result in higher cortisol availability and therefore glucocorticoid receptor activation. It is also reported that the activity of this enzyme is significantly elevated in the fatty tissue from obese humans and rodents.[131; 132; 145; 157]

Glucocorticoids also are able to influence the action and release of insulin, another endocrine adiposity signal and produce diabetogenic effects. Cortisol has been shown to directly inhibit insulin secretion from pancreatic beta cells and impair insulin initiated translocation of the intracellular glucose transporter (GLUT4) eventually leading to insulin resistance. Insulin resistance appears to contribute to hypertension through different mechanisms, such as vascular damage caused by chronic abnormalities in lipid and glucose metabolism; moreover, insulin resistance results in excess levels of circulating insulin in the body and a greater insulin response to glucose overload, observed in obese subjects. Insulin can cause sympathoactivation of different tissues, including the kidney, and may result in a modest increase in renal tubular sodium reabsorption and tissue renin–angiotensin–aldosterone system activation. However it appears this increased of activation of symphatic nervous system is not related to a corresponding rise in blood pressure and it has been suggested that insulin resistance may contribute to hypertension through other mechanisms such as abnormalities in lipid and glucose metabolism.[92; 145; 150]

1.11 Current project

Previous findings from our group provided evidences of the presence of common genomic determinants among group of related traits. For instance, following systematic genome wide linkage analysis of 213 blood pressure, anthropometric, and metabolic traits in a cohort of 120 French Canadian families from Saguenay–Lac St-Jean region of Quebec, clusters of overlapping quantitative-trait loci were found on several chromosomes including blood pressure QTLs co localized with renin and sodium QTLs on chromosome 3 and anthropometric trait QTLs on chromosome 1 that co localized with insulin levels QTLs; [27] moreover, the identified cluster on chromosome 1 overlapped with a cluster of triacylglycerol and blood pressure QTLs in rats[158] and open-field emotional reactivity in mice.[113] In another study, FTO gene, a well-replicated obesity locus, was found in two independent samples, not only related to obesity and insulin resistance, but also associated to hypertension.[187]

Current genome-wide scans are normally analyzing different phenotypes in isolation and ignore genes that are showing pleiotropic effect and important to the pathogenesis of correlated human traits. Identification of such pleiotropic genes can provide several benefits including identification of mechanistic links among related disorders, influencing the way disorders are grouped and classified; moreover, detection of such genes can expand the application of drugs for; example, if two disorders are as a result of abnormalities in the same biological process then the medication that is used for one can be examined for the other.[11; 159-161]

Motivated by above reasons, we aimed to investigate for shared genomic factors of habitual alcohol, tobacco and coffee use, response to mental and physical stress, obesity-related anthropometric traits and heart rate and blood pressure measurements. Alcohol, tobacco and coffee are the most commonly consumed psychoactive substances in the world; their concurrent use has been consistently shown across a wide variety of

populations.[41] Stress has been found both in experimental and clinical research to relate to initiation, intensification and relapse to substance use;[47; 53] besides, both stress and substance use are considered as contributing factors to the development of cardiovascular disease and cardiovascular risk factors including obesity.[52; 87; 93; 162; 167]

HPA axis, the main component of body's response to stressors has been implicated in substance use, obesity and regulation of cardiovascular system;[44; 46; 62; 92; 109] moreover, number of genes having function in HPA axis have been found to connect these traits, for instance, the mu opioid receptor (OPRM1) has been found to influence response to stressors, food intake and substance use;[46; 62; 87] however, like other polygenic traits, substance use, obesity, stress and cardiovascular traits have multifactorial etiology with a substantial complex genetic component and further studies are required to uncover the genetic nature and relations among these traits; therefore, we hypothesized that the links among substance use habits, obesity, stress and related cardiovascular outcomes may be in part due to shared genetic factors.

To investigate this hypothesis, we performed genome-wide linkage and association for genomic determinants of habitual alcohol, coffee and tobacco use, obesity-related anthropometric data, cardiovascular components of the behavioral response to mental stress of mathematical tests, changes in plasma cathecolamines after orthostatic test as the biomarkers of responses to mental and physical stress as well as 24-hour heart rate and blood pressure data in a cohort of 119 families from founder population of SLSJ who have been previously recruited to identify the genetic factors of hypertension;[27] furthermore, because sex and hypertension were found to explain significant portions of variations of these traits, the analyses were followed by sex and hypertension specific linkage and association analysis, we also subjected the candidate gene list driven from genetic analysis to functional annotation using the growing knowledge of gene information to explore the underlying biological meanings.

In addition, inclusion of former smokers in this study prompted us to investigate whether the observed correlation between tobacco use and obesity, hemodynamic and stress

related traits roots in environmental effect (smoking itself) and/or the influence of underlying genetic factors; therefore, to investigate this hypothesis, we probed the phenotypic and genotypic relatedness of tobacco use with hemodynamic, obesity and stress related traits by picking the smoking initiation and persistence phenotypes as well as significantly correlated traits with them and subjecting these phenotypes to univariate and bivariate family-based genome wide scans.

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Article1

Genetic analysis of habitual substance use, obesity-related traits, response to mental and physical stress and heart rate and blood pressure data revealed shared genes overrepresented in synapses

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2.1 Abstract

Links among substance use habits, obesity, stress and related cardiovascular outcomes may be in part due to shared genetic factors. To investigate this assumption, we performed genome-wide linkage and association tests for genetic components of habitual alcohol, tobacco and coffee use, response to mental and physical stress, obesity-related anthropometric traits and heart rate (HR) and blood pressure (BP) measurements in 119 multigenerational French Canadian families from Saguenay–Lac St-Jean population using 58000 SNPs and 437 microsatellite markers and followed with functional annotation on resulted genes. Current tobacco users were slender compared to former and never smokers. Habitual alcohol and/or tobacco users showed significantly attenuated HR response to mental stress as compared to non users; besides, hypertensives had stronger HR and SBP response to mental stress. We found shared genes associated / linked to substance use, obesity-related traits, response to mental and physical stress and HR and BP data including *CAMK4*, *CNTN4*, *DLG2*, *DAG1*, *FHIT*, *GRID2*, *ITPR2*, *NOVA1*, *NRG3* and *PRKCE* forming protein interaction network, involved in synaptic plasticity and highly expressed in brain related tissues. Pathway analysis on identified genes pointed (P = 0.03) to *Long-term potentiation pathway*. Specific subgroup analyses uncovered additional shared synaptic genes including *CAMK4*, *CNTN5*, *DNM3*, *KCNAB1* (Hypertension-specific), *CNTN4*, *DNM3*, *FHIT*, *ITPR1* and *NRXN3* (Sex-specific) having protein interactions with genes driven from general analysis; moreover, the results of pathway analysis and reported gene expression profiles of resulted genes from specific analyses revealed similar characteristics to those from general analysis. The substantial overlap among genomic factors of these traits supports the notion that the genetic variations in pathways of synaptic plasticity may be a common interface behind substance use, stress, obesity, HR, BP and the observed sex- and hypertension-specific genetic differences.

Keywords: genetic mapping, substance use, obesity-related traits, mental stress, physical stress, heart rate, blood pressure, synapse

2.2 Introduction

Alcohol, tobacco and coffee are among the most commonly consumed psychoactive substances in the world; their concurrent use has been consistently shown across a wide variety of populations.[1] Stress has been found both in experimental and clinical research to relate to initiation, intensification and relapse to substance use;[2] besides, both stress and substance use are considered as contributing factors to development of cardiovascular disease and cardiovascular risk factors including obesity.[3-5; 7]

Substance use, obesity, stress and cardiovascular disorder are complex traits having multifactorial etiology with a substantial genetic component.[4; 6; 7] HPA axis, the main component of body's response to stressors is also implicated in substance use, obesity and cardiovascular disorder; [8-12] for instance, the mu opioid receptor (OPRM1), a regulator of HPA axis, has been found to influence response to stressors, food intake and substance use;[3; 8; 12] however, the genetic nature and relations among these entities are still largely elusive and further studies are required.

We hypothesized that the connections among substance use habits, obesity, stress and related cardiovascular outcomes may be in part due to shared genetic factors. To investigate this hypothesis, we performed genome-wide linkage and association analysis for genomic determinants of habitual alcohol, coffee and tobacco use, obesity-related anthropometric data, cardiovascular components of the behavioral response to mental stress of mathematical tests and changes in plasma catecholamines after orthostatic test as the biomarkers of responses to mental and physical stress as well as 24-hour heart rate (HR) and blood pressure (BP) data in a cohort of 119 families from founder population of SLSJ that have been previously recruited to identify the genetic factors of hypertension;[13] furthermore, because sex and hypertension appeared to explain significant portions of variations of these traits,[19] the analysis were followed by sex and hypertension specific linkage and association analysis, we also subjected the identified candidate genes to functional annotation using the growing knowledge of gene information to explore the underlying biological meanings.

2.3 Methods and Procedures

2.3.1 Family cohort

Families were selected from the population of SLSJ region located in northeastern Quebec which is representing one of the largest founder populations in North America.[13; 14]

Founder effect provides several benefits for mapping the genomic determinants of polygenic disorders; for instance, allelic and locus heterogeneity, key features of common complex diseases that can obscure the association signals within disease-associated genomic regions are lower in founder population compared to general populations, the likelihood of population stratification that introduces errors and bias the results is also low in a founder population and the large size of LD blocks in a founder population reduces the number of markers for whole genome scan studies;[13; 15] moreover, the availability of extensive genealogical records of this population which is dating back to the original 17th-century settlers provides additional benefits for genetic studies.[13]

Details about families and extensive phenotyping have been described previously.[13; 16-19] In summary, families with catholic French Canadian origin were ascertained by the presence of at least one sib pair between the ages 18 to 55 years with hypertension and dyslipidemia. Additional selection criteria were the absence of (1) secondary hypertension, (2) diastolic blood pressure (DBP) >110 mmHg and the use of BP-lowering medications (3) body mass index (BMI) $\geq$ 35 kg/m^2 (4) diabetes mellitus (5) renal dysfunction (6) liver disease, (7) malignancy, (8) pregnancy, and (9) substance abuse, including alcohol. Once affected sib pairs were selected, all first- and second-degree relatives aged >18 years were invited to participate in the study, independent of health status. The recruited population included 119 families (average generations of 2.58) comprising 897 subjects and 1617 sib pairs.

The Research Ethics Committee of Complexe Hospitalier de la Sagamie (Chicoutimi, Quebec), Université du Québec à Chicoutimi, and the Centre hospitalier de l'Université de Montréal reviewed and approved the study. Written consents of all subjects were obtained prior to the commencement of data collection.

2.3.2 Phenotyping

Phenotyping for habitual substance use was carried out using questionnaires; individuals were asked about alcohol, coffee or tobacco use during clinical interviews. Those using a substance on regular basis were grouped as affected and those who never or occasionally use a substance were considered as unaffected; moreover, for non tobacco users, the information about their former status of tobacco use was also collected.

HR and BP changes to the mental stress of mathematical tests were used as markers of response to mental stress. The subjects were asked to sit for 10 minutes before the test; next, they passed 2 minutes arithmetic test. In parallel, HR, diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured every 5 minutes before the test (3 times), for every 2 minutes during the test (2 times) and every 2 minutes after the test (6 times). Stress response was defined as the differences in the first HR and BP values of math test and the average values of resting position before the test. The difficulty of test was increased at each level and the test was adjusted to assure some failure for all subjects.

During an orthostatic stress test, the plasma epinephrine (EP) and norepinephrine (NE) levels were monitored in blood samples taken from subjects once during 60 minutes supine and 10 minutes standing position; these data were used as biomarkers of response to physical stress.

Phenotyping for obesity consisted of 3 global and 11 regional measurements.[53] The global measures included BMI as well as total body fat derived from skinfold measurements and determined by bioimpedance (RJL Systems Inc). The regional measures included 5 skinfolds (biceps, triceps, subscapular, suprailiac, and thigh) as well as 6

extremity circumferences (upper arm, waist, hip, proximal thigh, middle thigh, and distal thigh).

BP and HR were measured every 20 minutes during the day and every 45 minutes during the night with an Accutracker II monitor (Sun Tech Medical Instruments, Inc.) for 24 hours.[13] Using these data, the pulse pressure (PP) was defined as the difference between SBP and DBP and mean arterial pressure (MAP) was defined as one third SBP plus two third DBP.

The inverse normal transformation was subsequently done on quantitative phenotype data to ensure a normal distribution prior to the analysis.

In addition, since significant portions of variation of studied traits were attributed to sex and hypertension status therefore general analysis was followed by sex- and hypertension-specific analysis to identify the candidate genes. We assumed specific subgroup analysis can minimize heterogeneity and helps to identify the specific genomic factors that would remain obscured following statistical adjustment. In general and hypertension specific groups, age and sex were included as covariates and in sex specific analysis, only age was set as covariate.

2.3.3 Phenotypic correlation tests

The generalized estimating equation (GEE) approach implemented in the GNU R statistical package version 2.6.1 was used to perform correlation tests which accounts for familial correlation via a sandwich estimator of the variance under exchangeable correlation. [20]

Unlike most standard statistical tests for correlation such as unpaired t-test, simple linear regression or the chi-square test that assume each of the subjects in a data set is independent of the others; GEEs use the generalized linear model to estimate more efficient and unbiased regression parameters relative to ordinary least squares regression in part

because they allow specification of a working correlation matrix that accounts for the form of within-subject correlation of responses on dependent variables of many different distributions.[21]

2.3.4 Estimation of heritability

Heritability estimates were done in whole cohort and next separately in males and females as well as hypertensives and normotensives using Sequential Oligogenic Linkage Analysis Routines (SOLAR) software, version 4.2.0.[22] SOLAR uses likelihood ratio tests to evaluate heritability by comparing a purely polygenic model with a sporadic model and also allows for covariate adjustments.

2.3.5 Genotyping

Genotype information has been previously described.[13; 18; 19] In summary, 469 subjects genotyped with GeneChip® Human Mapping 50K Array Xba240 (Affymetrix) and 537 subjects genotyped with 437 microsatellite markers. Altogether, 719 subjects genotyped using microsatellites and or GeneChip® Human Mapping 50K Array Xba240 (Affymetrix) were analyzed in present study.

Possible genotyping errors were detected and filtered (~ 0.2 %) using MERLIN package version 1.1.0.[23] An exact test of Hardy-Weinberg equilibrium (HWE)[24] on a subsample of unrelated individuals was performed using PEDSTATS program implemented in MERLIN package to remove genotypes that deviate from Hardy-Weinberg equilibrium (HWE). Minor allele frequencies (MAF) and linkage disequilibrium (LD) among SNPs were calculated using PLINK software version 1.06.[25] SNPs with $r^2 \leq 0.8$, HWE > 0.001 and MAF > 0.05 were included in analysis. The WGAviewr software version 1.52Z was used to determine the nearby genes around each SNP by specifying up- and down-stream span of 500 kbp.[26]

2.3.6 Genetic analysis

2.3.6.1 Linkage analysis

Because the haplotypic relationship between stable markers (e.g. SNPs) and potentially unstable but highly informative markers (e.g. microsatellites) indicates that LD might be maintained over considerable genetic distance in non-African populations;[27] hence, to increase the power of analysis microsatellites and SNPs genotype information were merged within a single database and haplotype map was created by specifying $r^2 > 0.4$.[28]

Multipoint linkage analysis was carried out on the haplotype map using MERLIN software package, version 0.10.2. MERLIN is based on the Lander-Green algorithm in which each alternative gene flow pattern in a pedigree is considered separately.[23]

For the qualitative phenotypes, multipoint linkage analysis was carried out using the non-parametric linkage (NPL) approach implemented in MERLIN.[23] which uses the Whittemore and Halpern NPL statistics to test for allele sharing among affected individuals and calculates the LOD score using the Kong and Cox linear model.

For quantitative phenotypes, after adjustment for covariates multipoint linkage analysis was performed on the haplotype map using the pedigree-wide regression analysis approach implemented in MERLIN,[29] this approach combines the simplicity and robustness of regression-based methods and the generality and greater power of variance-components models. It is based on a regression of estimated identity-by-descent (IBD) sharing between relative pairs on the squared sums and squared differences of trait values of the relative pairs

2.3.6.2 Association tests

The multipoint linkage analysis was followed by Family-Based Association Test (FBAT) implemented in the FBAT software version v2.0.2c that tests for association in the presence of linkage and reduces false positive associations consequently.[30]

SNPs with association $P < 10^{-3}$ were preselected for next steps. To identify specific genes for each trait, we selected loci that were within 1 LOD-drop interval around the linkage peak and had at least two identically annotated SNPs; furthermore, we corrected the P-values of SNPs by the number of SNPs within the area (Bonferroni correction) and excluded the non significant ones ($P < 0.05$). For commonly associated SNPs to more than one trait, combined P-values were calculated using Fisher's combined probability test[31] and P-value less than 1.4×10^{-6} was considered significant after Bonferroni correction by the number of SNPs analyzed (34741).

2.3.7 Functional annotations

Molecular interaction data were extracted from Ingenuity Pathway Analysis database (Ingenuity® Systems, www.ingenuity.com version 8.0) in which each gene interaction is supported by at least one reference from the literature, textbook, or canonical information stored in the Ingenuity Pathways Knowledge Base.

DAVID Functional Annotation Tool (http://david.abcc.ncifcrf.gov version 6)[32; 33] was used to identify significantly enriched KEGG pathways in the list of queried genes driven from genetic analysis.

Since genes that are having function in a biological process are expected to display higher expression and similar expression profiles in tissues that the biological process takes place. We analyzed the gene expression profiles of the shared genes across 65 normal human tissues using data from COXPRESdb database (http://coxpresdb.hgc.jp version c3.1)[34]. Hierarchical clustering of gene expression data was done using GenePattern software version 3.1.1[35] based on the complete Linkage method and Pearson correlation coefficient as the measure of distances.

The synapse databases, SynDB (http://syndb.cbi.pku.edu.cn release 2006) and G2Cdb (http://www.genes2cognition.org version 07) were used to query whether a gene

has synaptic function.[36; 37] Cytoscape software version 2.6.3 was used to build and visualize the networks.[38]

2.4 Results

2.4.1 General characteristics of phenotypes

Coffee use (79.2%) was more frequent compared to alcohol (26.5%) and tobacco use (26.1%); besides, while the between-sex difference was not significant in coffee use ($P = 0.3$) and smoking persistence ($P = 0.4$), the prevalence of habitual alcohol use, former smoking and current smoking were significantly higher in males compared to females (all $P < 0.04$); in addition, the prevalence of hypertension was significantly higher ($P = 0.02$) in never tobacco users as compared to former and current tobacco users (Table 1). Although alcohol and tobacco use decreased with aging, prevalence of habitual coffee use increased (all $P < 0.01$; Table 1).

Analysis of both global and regional obesity-related phenotypes indicated that hypertensive subjects are more obese than normotensives (Table 2). The catecholamine levels including epinephrine and norepinephrine were not significantly different between hypertensives and normotensives; however, hypertensives had stronger HR ($P = 0.03$) and SBP ($P = 0.02$) response to mental stress than normotensives; meanwhile, hemodynamic traits were significantly higher in hypertensive subjects compared to normotensives (Table 2).

Most of the studied traits display sexual dimorphism and were significantly different between females and males. Females had lower BMI (kg/m^2) compared to males (Mean±SE; 26.4±0.3 vs. 27.3±0.2, $P < 0.001$) and higher body fat (%) as determined by skinfold (Mean±SE; 37.9±0.4 vs. 25.5±0.3, $P < 0.001$) and bioimpedance (Mean±SE; 34.3±0.6 vs. 23.1±0.4, $P < 0.001$) as well as higher skinfold in biceps, triceps, subscapular, suprailiac, and thigh (Table 2). While BP values were significantly higher in males, females

had higher HR values compared to males. Males had higher epinephrine level; however, norepinephrines as well as cardiovascular responses to mental stress were not significantly different between males and females (Table 2).

BMI, body fat percentage determined by bioimpedance, waist circumference, waist-hip ratio and hip-thigh proximal ratio increased with aging while thigh circumferences decreased, EP levels also decreased with aging; in addition, while BP related traits increased with aging HR changes were insignificant (Table 2).

2.4.2 Phenotypic correlation results

Alcohol and tobacco users had attenuated HR response to mental stress compared to non users (both $P = 0.04$; Table 3). Most of the global and regional measurements of obesity were significantly lower in current tobacco users compared to former and never tobacco users suggesting an invert relation between obesity and tobacco use (Table 3); however, the Hip to Thigh Proximal ratio was higher in tobacco users compared to non users ($P = 0.001$). This appears to be in part due to significantly reduced thigh circumferences in tobacco users. In fact, the most significantly correlated trait to tobacco use in this study was thigh circumference. All three measures of thigh circumferences were prominently lower in tobacco users compared to never tobacco users with P-values ranging from 10^{-4} to 5×10^{-7} (Table 3). Former tobacco users also had significantly reduced thigh circumferences than never tobacco users; however, compared to current tobacco users they had higher ($10^{-2} \leq P \leq 10^{-4}$) thigh circumferences (Table 3).

While the NE levels were not significantly different among tobacco use statuses, tobacco users had higher EP compared to former and never tobacco users; meanwhile, EP levels were not significantly different between former and never tobacco users suggesting use of tobacco increases the level of EP in the body (Table 3).

Coffee use appears to increase mean ambulatory HR ($P = 0.01$). Tobacco users had lower mean ambulatory DBP compared to never ($P = 0.02$) and former tobacco users ($P =$

0.02) while the differences in mean ambulatory SBP and HR among tobacco use statuses were insignificant (Table 3).

2.4.3 Heritability estimates

The most heritable trait in our study was mean ambulatory HR (H2r = 64%); although, the between sex differences was small we found this trait to be almost completely attributed to genetic factors in normotensives (H2r = 99%) but not in hypertensives (H2r = 0.0%), the heritability of average sitting HR and sleep HR also displayed similar patterns (Table 4).

The heritability data indicated that both initiation (61% in former vs. never tobacco users) and persistence of tobacco use (46% in current vs. former tobacco users) are highly attributed to genetic factors; sex-specific heritability estimates for tobacco use showed that genetic factors are more important in initiation (males = 81%, females = 56%) and persistence of tobacco use (males = 97%, females = 23%) in males compared to females; however, the heritability of alcohol use in males was lower compared to females (Table 4), we also observed a maternal effect on alcohol use behavior that in case parents are non habitual alcohol users, 27% of sibs (16 users vs. 43 non users) acquire alcohol use habit and if father is alcohol user, 44% of sibs (20 users vs. 14 non users); however, in case mother is alcohol users, 75% of sibs (3 users vs. 9 non users) gain the habit.

2.4.4 Genetic findings and functional annotation

2.4.4.1 General analysis

Table 5 lists candidate loci within 1 LOD-drop interval around the linkage peaks. In a common area of linkage for HR response (LOD = 2.8) and tobacco use (LOD = 2.6) on chromosome 12, we found SNPs inside ITPR2 gene associated to these traits as well as

supine NE (Figure 1 and Table 6); moreover, multivariate association analysis showed that these SNPs are significantly associated to all these traits ($P \leq 0.003$).

Joint linkage and association analysis also uncovered SNPs inside genes; LRP1B, GRID2, TLL1, PCM1, HTR2A, NETO1 and nearby AGTR1 and within intergenic regions between LPHN2 and TTLL7, COL24A1 and ODF2L, DAB2 and PTGER4, CHRM2 and MTPN, and NETO1 and CBLN2 (Table 5). Probing the protein interactions revealed that GRID2, HTR2A, LPHN2, LRP1B, MTPN and NETO1 are sharing interactions with synaptic protein, DLG4 (Figure 3) and searching the synapse databases indicated CHRM2, DAB2, GRID2, HTR2A, ITPR2, LPHN2, NETO1 genes have synaptic function (Table S1).

Family based association tests identified genes commonly associated at the level of $P < 0.05$ after correction for multiple testing among substance use, obesity, stress responses and hemodynamic traits (Table 6). GRID2, ITPR2, LRP1B and PCM1 genes found under the linkage peaks were associated to other traits as well (Table 5 and 6). Functional annotation information showed that 44% of the shared genes are having synaptic function (Table S1) and the results of KEGG pathway analysis on the shared genes using David functional annotation tool significantly pointed to Long-Term Potentiation pathway ($P = 0.03$).

Next we checked the expression of these commonly associated genes across 65 human tissues using data from COXPRESdb database. The gene expression results were in agreement with other functional annotation information. We found, identified genes are having higher expression in a cluster of brain-related tissues including Brain lobes, Cerebral cortex, Amygdala, Hippocampus and Putamen; besides, unlike other tissues, genes in each group tend to display similar expression profiles in these tissues (Figure 2 and Table 9).

2.4.4.2 Sex-specific and hypertension-specific analysis

Hypertension-specific genetic analysis uncovered SNP, rs4687150 (HWE $P = 0.3$, MAF = 0.3) inside IL1RAP gene associated to coffee use with negative association signal

(Z = -3.5, P = 0.0004) in hypertensives and positive association signal (Z = +3.8 P = 0.0002) in normotensives. Sex-specific genetic analysis revealed SNPs, rs4888197 (HWE P = 1, MAF = 0.2) inside PLCG2 gene associated to thigh skinfold and rs847936 (HWE P = 0.7, MAF = 0.8) near SCIN gene associated to tobacco use with negative association signals (both P ≤ 0.0009) in females and positive association signals (both P ≤ 0.0009) in males. Interestingly, we found, all three genes; IL1RAP, PLCG2 and SCIN share synaptic function (Table S1); meanwhile, SNP, rs847936 was not only positively associated to never smokers in males but also was positively associated to both wake HR (P = 0.00095) and sleep HR (P = 0.000055) in males (Table 8).

Under a common area of linkage for body fat percentage by impedance in both male-specific (LOD = 2.0) and hypertension-specific analysis (LOD = 2.8), we found SNPs inside DNM3 gene associated to these traits (Table 7); moreover, DNM3 along ADAMTS3, CDH12, DTNBP1, KCNAB1, KIF5B, RGS4 and SEMA6A genes driven from joint linkage and association shared synaptic function (Table S1). CAMK4, CNTN4, CSMD1, FHIT, OLFM4, PTPRD and RORA genes driven from general analysis were also appeared in specific results; besides, RORA gene that was identified under the linkage peak associated to waist-hip ratio in normotensives was associated to standing EP in this group as well. Shared genes, BAI3, CNTN4, DCLK1, FHIT, INHBA, ITPR1, NLGN1, NRXN3, PTPRR and SCIN driven from sex-specific analysis and CAMK4, CNTN5, GLRA3, PTPRD, SPINK5 driven from hypertension specific analysis had synaptic function (Table 8 and S1).

Pathway analysis using commonly associated genes driven from sex-specific and hypertension-specific analysis once more significantly pointed to Long-Term Potentiation (P = 0.02) and GnRH signaling pathways which are interconnected pathways (P = 0.04).

2.4.4.3 Gene expression profiles

The gene expression data of shared genes driven from general and specific analysis are presented in Table 9. We found that similar to shared genes driven from general

analysis commonly associated genes identified through either sex-specific or hypertension-specific analysis also have higher expression in a cluster of brain tissues including Brain lobes, Cerebral cortex, Amygdala, Hippocampus and Putamen (Table 9).

2.4.4.4 Protein interactions

Candidate genes driven from general (n = 76), sex-specific (n = 31) and hypertension-specific analysis (n = 38) were searched in the Ingenuity database and a connectivity diagram was built on the basis of interactions. Figure 3 shows an overview of these interactions. Although these genes were driven from different sets of analysis, we found numerous interactions among these genes. In the constructed network, there were 45 (59%) genes identified through general analysis, 18 (47%) genes identified through hypertension specific and 20 (65%) genes identified through sex-specific analysis.

We also checked for associations of SNPs encompassed in the missing genes of network (Figure 3). In this manner, we retrieved SNPs; rs6938572 (P = 0.0006) and rs2327017 (P = 0.0003) upstream BMP6 gene associated to average sitting DBP; rs2370413 (P = 0.0003) and rs2887780 (P = 0.0004) inside CACNA1C gene associated to skinfold subscapular in males and SBP response to mental stress respectively; rs10492133 (P = 0.0009) inside GRIN2B associated to tobacco use; rs10491321 (P = 0.00035) upstream and rs7705319 (P = 0.0006) inside PPP2CA gene associated to tobacco; SNPs, rs953944 (P = 0.0007) rs1029819 (P = 0.0008) associated to tobacco use and rs10485912 (P = 0.00075) associated to overall SBP inside MAGI2 gene; SNP, rs93059 inside NFKB1 gene associated to average sitting HR (P = 0.00098) and DBP response to mental stress (P = 0.0006); and SNPs inside ITPR1 gene associated to ($0.0004 \leq P \leq 0.0007$); SBP, DBP and SBP response to mental stress.

2.5 Discussion

In this study, we have investigated the connections among substance use, obesity, response to mental and physical stress and hemodynamic traits. The current research project is based

on French population of Saguenay-Lac-St-Jean region located in northeastern Quebec[13] which is representing one of the largest founder populations in North America with about 300,000 inhabitants.[39] [14] Founder effect provides several benefits for mapping the genomic determinants of polygenic disorders including limited allelic and locus heterogeneity, low likelihood of population admixture and stratification, large size of LD blocks as well as good genealogical records.[13; 15; 16]

Tobacco users had lower BP and obesity as well as reduced response to mental stress; while, hypertensive subjects were more obese and had stronger HR and SBP response to mental stress. These findings suggest tobacco use may decrease the BP in several ways.

Since, obesity increases the risk of hypertension;[7] therefore, the observed inverse correlation between obesity and tobacco use in our study partly explain the lower BP among tobacco users. In addition, stress is related to hypertension; for instance, It has been reported that high cardiovascular response to environmental stressors which also observed in this study, is a predictor of hypertension;[2; 3] thus, the finding that habitual tobacco users showed significantly attenuated HR response to the mental stress as compared with non users suggest the damping effect of tobacco use on response to environmental stress may also account for the lowering effect of tobacco use on BP.

In fact, the relief from stress and negative affections and enhancing the positive moods are among the main reasons for substance use.[40; 41] Physiological studies have also shown that both substance use and stress influence the synaptic plasticity and substance use can change the sensitivity of synaptic plasticity to stressors,[42; 43] for instance it has been reported that concurrent chronic nicotine treatment and stress prevents stress-induced impairment of Long-Term Potentiation pathway. [44; 45]

Obesity and addiction also appear to share common neurological underpinnings; earlier studies reported that obesity and drug addiction may in part mediated by persistent changes in neural circuits.[46; 47] In fact, similar to psychoactive drugs, palatable food can activate the brain reward system and pharmacological blockade of, or experimental damage

to forebrain dopamine systems attenuates free feeding and lever-pressing for food reward, as well as the rewarding effects of psychoactive substances. Synaptic plasticity also appears to be involved in the regulation of energy homeostasis and is acting as an important path through which peripheral metabolic hormones influence brain functions.[48]

Hypertension is in part a centrally mediate trait, in fact central nervous system (CNS) is considered as initial source of blood pressure elevation (BP). For instance, once brain recognized a stressful stumuli, information about stress from the prefrontal cortex is transmitted to the hypothalamic defense area through dopaminergic neurons. An increase in dopaminergic neuron activity evokes the defense response. This is called synaptic sensitization which is an attribute of thalamocortical and memory neurons; synaptic sensitization has been implicated in both stress-and-salt-related hypertension and obesity associated hypertnesion and it ensures that repeated stimulation of the defense pathway makes it respond to ever milder stresses, so that hypertension eventually becomes permanent.[7]

Consistent with these findings, genetic analysis uncovered numerous shared genes among substance use, obesity, response to mental and physical stress and HR and BP data including CAMK4, CNTN4, CSMD1, CTNNA2, DGKB, DLG2, FHIT, GRID2, ITPR2, LRP1B, NOVA1, NRG3, PCM1, PRKCE, RAP1B and RORA sharing synaptic function; furthermore, the result of gene-gene interactions indicate that these genes sharing protein interactions and tend to form protein network; pathways analysis also pointed to Long-Term Potentiation pathway which is an important form of synaptic plasticity.

In agreement with these results; gene expression findings also revealed that these shared genes have higher expression in a cluster of brain related tissues including Temporal, Occipital, Frontal and Parietal lobes, Cerebral cortex, Amygdala and Hippocampus; besides, in these tissues majority of identified genes tend to display similar expression profiles unlike other tissues. Overall, these findings indicate that the common interface among these traits is likely synaptic related process.

The sex-specific and hypertension-specific genetic analysis uncovered IL1RAP, PLCG2 and SCIN genes that were significantly negatively associated to a trait in one group and significantly positively associated to the same trait in the contrary group. All three genes shared synaptic function. Consistent with the finding of SCIN gene for tobacco use, it is reported that nicotinic-receptor stimulation induces the redistribution of SCIN protein.[49]

The results of pathway analysis and gene expression profiles of commonly associated genes driven from sex-specific and hypertension-specific analysis showed similar characteristics to general results; moreover, we found interactions and common genes among gene sets driven from general analysis and sex and hypertension-specific analysis, suggesting sex-specific and hypertension-specific genetic differences appear to be due to variations in similar biological process identified through general analysis.

Based on these findings, we propose a model that (Figure 4) once an environmental stress is perceived at synapses, it can alter the efficacy of synaptic plasticity; however, other environmental factors as substance use can modify the sensitivity of synaptic plasticity to the stress. In the long term, the interaction between genetic make-up of synapses with environmental factors can shape individuals's life styles and habits; in the other side, the taken habits and lifestyle influence the body's systems including cardiovascular system and affect the cardiovascular outcomes as well as cardiovascular risk factors including body weight; nonetheless, similar to different routes ended to the same location, there are other factors that influence the cardiovascular system through other mechanisms. Our results also suggest that synaptic plasticity may be a common interface behind many of other complex disorders in which life style is a contributing factor and further support the notion of human disease network.[50-52]

This study is limited on genomic coverage; however, the large size of LD blocks in this founder population reduces the number of markers for genome wide scan.[13; 15; 54] Small sample size and lack of replication are also important potential issues in genome wide scan studies. Some common risk variants cannot be feasibly detected in small cohorts; moreover

the detected variants may not be reproducible in other cohorts which points to presence of locus heterogeneity.[55] Considering that SNPs and genes carry out their functions through intricate pathways, to address this issue, we did network-based genetic analysis which aims to determine whether the variations that are more strongly associated to a phenotype tend to significantly cluster in a biological process.

Identification of shared genetic factors among the studied traits suggests that complex phenotypes may not be completely unrelated and provide implications for design of gene-mapping studies that jointly examine complex traits, specifically those traits that are correlated. Current study also provides a perspective on how large human genomic data can be processed to implicate the role of biological processes that otherwise would be left unrecognized. The substantial overlap among genomic determinants of substance use, stress, obesity and hemodynamic traits in synaptic plasticity related processes calls for additional studies on plausible influence of synaptic plasticity on shaping life style habits and physiological outcomes.

Author Contributions

P.H., J.T., D.G., T.A.K. and A.W.C.J. established family cohort and conducted sample selection, phenotyping, genotyping and project management. P.H. is director of project and supervised the entire study. M.N. did the quality checks of phenotype and genotype data; calculated heritabilities; carried out statistical analysis, genetic scans, pathway/network analysis and, insilico-functional annotation and; drafted the manuscript. O.Š. and J.T. contributed to the conception and design of study.

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Table 1. Distribution of substance use traits by sex and hypertension status and their correlations[1] with sex, age[2] and hypertension[3]

Trait	Females				Males				Sex (females)		Aging		Hypertension (hypertensives)		Total
	Unaffected		Affected		Unaffected		Affected								
	Nor[4]	Hyp[5]	Nor	Hyp	Nor	Hyp	Nor	Hyp	Z	P	Z	P	Z	P	
Alcohol users vs. non users	126	190	44	17	84	113	72	52	-5.6	**< 1E-04**	-2.7	**0.004**	-2.0	**0.025**	698
Current vs. non tobacco users	117	174	50	36	99	125	57	39	-2.2	**0.02**	-3.7	**0.0001**	-3.4	**0.0003**	697
Never vs. ever tobacco users	85	102	82	108	106	114	50	50	5.5	**< 1E-04**	-1.9	**0.03**	2.0	**0.02**	697
Current vs. former tobacco users	35	66	50	36	49	75	57	39	0.3	0.4	-4.5	**< 1E-04**	-3.2	**0.0006**	407
Current vs. never tobacco users	82	108	50	36	50	50	57	39	-4.1	**< 1E-04**	-0.9	0.2	-3.0	**0.001**	472
Former vs. never tobacco users	82	108	35	66	50	50	49	75	-4.8	**< 1E-04**	3.4	**0.0004**	-0.6	0.3	515
Coffee users vs. non users	41	30	127	173	33	37	120	118	0.6	0.3	3.4	**0.0003**	-0.2	0.4	679

[1] Correlation test was done using GEE method. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa.
[2] Correlation model is substance use ~ sex + age.
[3] Correlation model is hypertension status ~ sex + age + substance use.
[4] Normotensives
[5] Hypertensives

Table 2. Descriptive statistics of quantitative traits distributed by sex, hypertension status and in entire cohort along their correlations[1] with sex, age[2] and hypertension[3]

Trait	Females				Males				Total		Sex (females)		Hypertension (hypertensives)		Aging	
	Normotensives		Hypertensives		Normotensives		Hypertensives									
	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	Z	P	Z	P	Z	P
BMI (kg/m2)	175	24.7±0.4	254	27.5±0.3	162	26.3±0.4	201	28.1±0.3	792	26.8±0.2	-4.9	**5.E-07**	5.4	**3.E-08**	2.7	**4.E-03**
Waist Hip ratio	171	0.8±0	251	0.8±0	161	0.9±0	202	1±0	785	0.9±0	-24.4	**7.E-132**	4.5	**3.E-06**	8.8	**5.E-19**
Waist circumference (cm)	172	80.1±1	251	87.7±0.9	161	92.9±0.9	203	99.3±0.8	787	90.1±0.5	-16	**1.E-57**	4.8	**1.E-06**	5.3	**6.E-08**
Hip circumference (cm)	171	99.2±0.8	251	102.9±0.7	161	98±0.5	202	100.8±0.5	785	100.6±0.3	2.1	**2.E-02**	3.8	**7.E-05**	1	2.E-01
Hip Thigh Proximal ratio	171	1.7±0	238	1.7±0	158	1.7±0	193	1.7±0	760	1.7±0	-4	**3.E-05**	-0.6	3.E-01	15.5	**1.E-54**
Thigh proximal circumference (cm)	171	59±0.6	238	58.8±0.4	159	57.2±0.5	194	58.1±0.4	762	58.3±0.2	4.1	**2.E-05**	3	**2.E-03**	-5.5	**2.E-08**
Thigh mid circumference (cm)	171	52.3±0.5	241	52.8±0.4	159	52.7±0.5	195	53.4±0.4	766	52.8±0.2	-0.8	2.E-01	4.1	**2.E-05**	-4.6	**2.E-06**
Thigh distal circumference (cm)	171	39.8±0.4	239	40.6±0.3	159	40±0.3	194	40.9±0.3	763	40.4±0.2	-0.8	2.E-01	3.2	**7.E-04**	-1.6	6.E-02
Skinfold bicep1 (mm)	160	21.9±1.1	202	23.7±0.8	154	16.4±1	180	16.8±1	696	19.9±0.5	6.2	**2.E-10**	4.4	**7.E-06**	1.2	1.E-01
Skinfold bicep2 (mm)	160	22.1±1.1	202	23.9±0.8	154	16.7±1	180	16.9±1	696	20.1±0.5	6	**1.E-09**	4.3	**7.E-06**	1.1	1.E-01
Skinfold bicep3 (mm)	160	22.3±1.1	201	24±0.9	154	16.7±1	180	16.8±1	695	20.1±0.5	5.9	**2.E-09**	4	**3.E-05**	1	2.E-01

[1] Correlation test was performed using GEE. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa.
[2] Correlation model is trait ~ sex + age.
[3] Correlation model is hypertension status ~ sex + age + substance use + trait.

Mean skinfold biceps (mm)	160	22.1±1.1	202	23.9±0.8	154	16.6±1	180	16.8±1	696	20±0.5	6	**7.E-10**	4.3	**1.E-05**	1.1	1.E-01
Skinfold triceps1 (mm)	161	30±1	202	34.1±0.8	154	23±1	180	23.8±1	697	28±0.5	8	**9.E-16**	4.1	**2.E-05**	0.9	2.E-01
Skinfold triceps2 (mm)	161	30.2±1	202	34.5±0.8	154	23.3±1.1	179	23.6±0.9	696	28.2±0.5	7.7	**6.E-15**	4.2	**1.E-05**	1	2.E-01
Skinfold triceps3 (mm)	161	30.4±1	201	34.5±0.8	154	23.2±1.1	179	23.6±1	695	28.2±0.5	7.7	**9.E-15**	4.1	**2.E-05**	1	2.E-01
Mean skinfold triceps (mm)	161	30.2±1	202	34.4±0.8	154	23.1±1.1	180	23.8±1	697	28.2±0.5	7.7	**5.E-15**	4.2	**1.E-05**	1	2.E-01
Skinfold subscapular1 (mm)	161	24±1	201	28.1±0.8	154	22.8±0.8	177	25.9±0.8	693	25.4±0.4	2.2	**2.E-02**	4.1	**2.E-05**	1.3	1.E-01
Skinfold subscapular2 (mm)	161	24.3±1	201	28.2±0.9	154	22.7±0.8	177	25.7±0.8	693	25.5±0.4	2.3	**1.E-02**	4	**4.E-05**	1.2	1.E-01
Skinfold subscapular3 (mm)	161	24.4±1	201	28.3±0.9	154	22.9±0.9	177	25.8±0.8	693	25.5±0.5	2.3	**1.E-02**	4	**3.E-05**	1.1	1.E-01
Mean skinfold subscapular (mm)	161	24.2±1	201	28.2±0.9	154	22.8±0.8	177	25.8±0.8	693	25.5±0.4	2.2	**1.E-02**	4	**3.E-05**	1.2	1.E-01
Skinfold suprailiac1 (mm)	159	23.7±1.1	201	26.9±0.7	153	21.6±1	177	24.8±0.9	690	24.5±0.5	3.1	**9.E-04**	3.8	**7.E-05**	-1	2.E-01
Skinfold suprailiac2 (mm)	159	23.7±1.1	201	27.2±0.7	153	21.9±1	177	24.8±0.9	690	24.6±0.5	3.2	**8.E-04**	4.1	**2.E-05**	-1	2.E-01
Skinfold suprailiac3 (mm)	159	23.8±1.1	201	27.2±0.7	153	21.9±1	177	25.1±0.9	690	24.7±0.5	2.9	**2.E-03**	4.1	**2.E-05**	-0.9	2.E-01
Mean skinfold suprailiac (mm)	159	23.7±1.1	201	27.1±0.7	153	21.8±1	177	24.9±0.9	690	24.6±0.5	3	**1.E-03**	4	**3.E-05**	-0.9	2.E-01
Skinfold thigh1 (mm)	145	39.2±1.1	191	43±0.9	149	26.9±1.3	175	24.3±1.1	660	33.5±0.6	13.2	**4.E-40**	2.5	**6.E-03**	-1.1	1.E-01
Skinfold thigh2 (mm)	144	39.2±1.1	191	43.3±0.9	149	27.1±1.3	175	24.5±1.1	659	33.7±0.6	12.3	**4.E-35**	2.5	**6.E-03**	-1	2.E-01
Skinfold thigh3 (mm)	144	39.5±1.1	191	43.6±0.9	148	27±1.3	175	24.6±1.1	658	33.9±0.6	13.4	**3.E-41**	2.7	**4.E-03**	-1.2	1.E-01
Mean skinfold thigh (mm)	145	39.4±1.1	191	43.3±0.9	149	27.1±1.3	175	24.5±1.1	660	33.8±0.6	12.9	**2.E-38**	2.6	**5.E-03**	-1.1	1.E-01
Body fat percentage bioimpedance	137	30.4±0.8	177	37.4±0.8	128	21.2±0.7	158	24.6±0.6	600	28.9±0.4	18.7	**5.E-78**	3.5	**2.E-04**	9.7	**1.E-22**
Body fat (%)	158	36.3±0.6	201	39.1±0.4	153	25±0.5	175	26±0.4	687	32±0.3	27.7	**3.E-169**	4.4	**5.E-06**	1	2.E-01

Supine EP (pg/ml)	66	41.9±2.1	40	40.5±2.6	57	43.3±2.3	49	46±3.2	212	42.9±1.3	-1.5	7.E-02	0	5.E-01	0.5	3.E-01
Standing EP (pg/ml)	68	50.5±3.3	37	38.6±2.4	54	57±4.7	52	54.9±3.7	211	51.2±1.9	-3.1	**1.E-03**	-0.7	2.E-01	-2.6	**5.E-03**
Response EP (pg/ml)	63	9.6±3.6	31	-1.1±2.8	54	13.8±4.6	43	6±4	191	8.2±2	-1	2.E-01	-1.2	1.E-01	-2.4	**9.E-03**
Supine NE (pg/ml)	73	171.7±7.1	48	167.3±9.8	57	169.4±8.6	60	160.2±9.5	238	167.4±4.3	0.7	2.E-01	-0.9	2.E-01	-0.2	4.E-01
Standing NE (pg/ml)	73	391.8±14.9	48	407.9±24.6	55	397.1±23.3	61	395.9±20.7	237	397.4±10.1	0.4	4.E-01	0.2	4.E-01	1.6	5.E-02
Response NE (pg/ml)	73	220.1±11.2	48	240.6±18.8	55	230.3±20.5	60	235.8±15.2	236	230.7±8	0	5.E-01	0.6	3.E-01	2.1	**2.E-02**
DBP response to mental stress (mmHg)	77	10±0.8	57	12.1±1	64	9.6±0.9	71	9.4±0.9	269	10.2±0.4	1.5	7.E-02	1.4	8.E-02	-1.3	1.E-01
SBP response to mental stress (mmHg)	77	12.1±1.3	56	18.4±1.6	64	14.9±1.4	71	19.5±1.6	268	16.1±0.7	-1.5	7.E-02	2	**2.E-02**	0.9	2.E-01
HR response to mental stress (beast/min)	78	7.8±1.4	57	9.4±1.1	64	7±1.1	71	8.5±1	270	8.1±0.6	0.7	2.E-01	1.8	**3.E-02**	-0.6	3.E-01
Average sitting DBP (mmHg)	77	69.2±0.9	57	79±1.4	64	75.1±1.1	69	86±1.3	267	77.1±0.7	-5.1	**2.E-07**	4.1	**2.E-05**	6.8	**4.E-12**
Average sitting SBP (mmHg)	77	109±1.3	57	136.8±2.6	64	118.1±1.3	69	136±2.1	267	124.1±1.2	-2.6	**4.E-03**	5.8	**4.E-09**	6.2	**3.E-10**
Average sitting HR (beast/min)	77	71.1±1.2	57	73.8±1.5	64	64.7±1.2	67	67.1±1.2	265	69.1±0.7	4.4	**6.E-06**	2.1	**2.E-02**	-1.5	6.E-02
Mean ambulatory DBP (mmHg)	61	67.4±0.8	43	79.3±1.5	48	73.3±1.1	51	84.7±1.2	203	75.6±0.7	-6.2	**3.E-10**	5.9	**2.E-09**	9.3	**5.E-21**
Mean ambulatory SBP (mmHg)	61	110.3±1	43	130.6±2.4	48	120.7±1.3	51	133.7±1.9	203	122.9±1	-5.3	**8.E-08**	5.4	**3.E-08**	5.6	**9.E-09**
Mean ambulatory HR (mmHg)	61	74.9±1	43	79.9±1.4	48	70.5±1.6	51	76.3±1.5	203	75.3±0.7	1.7	**4.E-02**	2.9	**2.E-03**	0.3	4.E-01
Sleep DBP (mmHg)	74	61.4±1	50	70.3±1.6	61	67.2±1.1	63	74.8±1.3	248	68±0.7	-5.8	**3.E-09**	3.8	**8.E-05**	5.5	**2.E-08**
Sleep SBP (mmHg)	74	102.5±1.1	50	117.6±2.4	61	112.6±1.2	63	122.2±2	248	113±1	-5.5	**2.E-08**	4.3	**1.E-05**	4.1	**2.E-05**
Sleep HR (beast/min)	74	71.2±1.2	49	74.8±1.4	60	65.8±1.3	63	67.3±1.6	246	69.6±0.7	3.4	**4.E-04**	1.2	1.E-01	0.3	4.E-01
Sleep MAP (mmHg)	74	75.1±1	50	86.1±1.8	61	82.3±1	63	90.6±1.4	248	83±0.7	-5.9	**2.E-09**	4.2	**1.E-05**	5	**2.E-07**

Sleep PP (mmHg)	74	41.1±0.7	50	47.4±1.6	61	45.4±0.9	63	47.4±1.4	248	45±0.6	-2.5	**6.E-03**	3.1	**9.E-04**	0.7	2.E-01
Wake DBP (mmHg)	73	71±0.9	49	79.6±1.6	62	75.5±1	63	84.4±1.3	247	77.2±0.7	-5	**3.E-07**	3.8	**8.E-05**	5.5	**2.E-08**
Wake SBP (mmHg)	73	115.3±1.2	49	131.3±2.5	62	124.4±1.3	63	136.5±1.9	247	126.2±1	-5.4	**4.E-08**	4.5	**4.E-06**	4.7	**1.E-06**
Wake HR (beast/min)	73	81.6±1	49	85±1.6	62	76.6±1.4	63	81.7±1.8	247	81±0.8	2.1	**2.E-02**	2.2	**1.E-02**	0.6	3.E-01
Wake MAP (mmHg)	73	85.8±0.9	49	96.8±1.7	62	91.8±1	63	101.8±1.4	247	93.6±0.7	-5.9	**2.E-09**	4.5	**4.E-06**	5.6	**1.E-08**
Wake PP (mmHg)	73	44.3±0.9	49	51.8±1.7	62	48.9±1	63	52.1±1.4	247	48.9±0.6	-2.8	**2.E-03**	2.9	**2.E-03**	1.6	6.E-02
Overall PP (mmHg)	61	42.9±0.7	43	51.3±1.6	48	47.4±0.9	51	48.9±1.2	203	47.3±0.6	-2.2	**1.E-02**	3.2	**7.E-04**	0.5	3.E-01

Table 3. Correlation[1] of obesity-related traits, stress responses and HR and BP data with substance use

Trait	Alcohol users vs. non users		Coffee users vs. non users		Current vs. non tobacco users		Current vs. never tobacco users		Former vs. never tobacco users		Current vs. former tobacco users	
	Z	P	Z	P	Z	P	Z	P	Z	P	Z	P
BMI (kg/m2)	-0.2	4.30E-01	0.3	3.70E-01	-3.5	**2.50E-04**	-3.3	**5.30E-04**	-0.8	2.20E-01	-3.1	**1.10E-03**
Waist Hip ratio	0.7	2.40E-01	-0.5	3.10E-01	-0.6	2.70E-01	-0.2	4.00E-01	0.5	3.10E-01	-1.1	1.40E-01
Waist circumference (cm)	-0.4	3.60E-01	-0.4	3.50E-01	-2.9	**2.10E-03**	-2.4	**8.30E-03**	-0.3	3.80E-01	-2.6	**4.40E-03**
Hip circumference (cm)	-1.6	5.50E-02	0.4	3.30E-01	-3.4	**3.10E-04**	-3	**1.20E-03**	-0.6	2.90E-01	-3	**1.50E-03**
Hip Thigh Proximal ratio	-0.9	2.00E-01	0.9	1.90E-01	3.1	**1.10E-03**	3.2	**6.10E-04**	2.7	**3.70E-03**	2	**2.60E-02**
Thigh proximal circumference (cm)	-0.8	2.20E-01	0	4.80E-01	-4.9	**4.00E-07**	-4.9	**5.50E-07**	-2.1	**1.90E-02**	-3.6	**1.30E-04**
Thigh mid circumference (cm)	0.2	4.10E-01	-0.1	4.60E-01	-4.2	**1.10E-05**	-3.7	**1.10E-04**	-1.4	8.50E-02	-3.6	**1.40E-04**
Thigh distal circumference (cm)	0.6	2.90E-01	0.8	2.30E-01	-3.6	**1.40E-04**	-3.9	**4.90E-05**	-1.8	**3.40E-02**	-2.3	**1.00E-02**
Skinfold bicep1 (mm)	0.6	2.90E-01	1.4	8.80E-02	-1.3	1.00E-01	-1.5	6.40E-02	-1.3	9.40E-02	-0.8	2.10E-01
Skinfold bicep2 (mm)	0.5	2.90E-01	1.3	1.00E-01	-1.3	1.00E-01	-1.5	7.10E-02	-1.2	1.20E-01	-0.9	2.00E-01
Skinfold bicep3 (mm)	0.5	3.20E-01	1.1	1.40E-01	-1.4	8.80E-02	-1.6	5.60E-02	-1.3	1.00E-01	-0.9	1.90E-01

[1] Correlation test was performed using GEE method. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa. Correlation model is trait ~ sex + age + substance use.

Mean skinfold biceps (mm)	0.5	3.00E-01	1.2	1.10E-01	-1.3	9.80E-02	-1.5	6.50E-02	-1.2	1.10E-01	-0.9	2.00E-01
Skinfold triceps1 (mm)	0	4.90E-01	0.1	4.80E-01	-1.8	**3.30E-02**	-1.9	**3.00E-02**	-0.9	1.80E-01	-1	1.60E-01
Skinfold triceps2 (mm)	0	4.90E-01	0.1	4.80E-01	-1.8	**3.70E-02**	-1.8	**3.40E-02**	-0.8	2.00E-01	-1	1.60E-01
Skinfold triceps3 (mm)	0	4.80E-01	0	4.90E-01	-1.7	**4.80E-02**	-1.6	5.80E-02	-0.7	2.40E-01	-1.1	1.30E-01
Mean skinfold triceps (mm)	0	4.80E-01	0.1	4.50E-01	-1.9	**3.00E-02**	-1.9	**3.10E-02**	-0.8	2.20E-01	-1.1	1.40E-01
Skinfold subscapular1 (mm)	-0.5	3.00E-01	0.3	3.70E-01	-1.6	5.30E-02	-1.6	5.30E-02	-0.7	2.50E-01	-0.8	2.10E-01
Skinfold subscapular2 (mm)	-0.6	2.70E-01	0.4	3.60E-01	-1.4	8.30E-02	-1.4	8.10E-02	-0.6	2.80E-01	-0.7	2.50E-01
Skinfold subscapular3 (mm)	-0.7	2.40E-01	0.5	3.10E-01	-1.7	**4.30E-02**	-1.7	**4.30E-02**	-0.7	2.60E-01	-0.8	2.00E-01
Mean skinfold subscapular (mm)	-0.6	2.80E-01	0.4	3.50E-01	-1.6	5.50E-02	-1.6	5.40E-02	-0.7	2.50E-01	-0.8	2.20E-01
Skinfold suprailiac1 (mm)	-0.8	2.20E-01	-0.2	4.30E-01	-1.8	**3.40E-02**	-2.3	**9.50E-03**	-1.6	5.80E-02	-0.7	2.30E-01
Skinfold suprailiac2 (mm)	-0.8	2.10E-01	0	4.90E-01	-2	**2.20E-02**	-2.3	**1.00E-02**	-1.3	1.00E-01	-1.1	1.30E-01
Skinfold suprailiac3 (mm)	-0.7	2.50E-01	-0.1	4.70E-01	-1.8	**3.40E-02**	-2.2	**1.50E-02**	-1.2	1.10E-01	-0.9	1.70E-01
Mean skinfold suprailiac (mm)	-0.7	2.30E-01	-0.1	4.60E-01	-1.9	**2.80E-02**	-2.3	**1.10E-02**	-1.4	8.00E-02	-0.9	1.80E-01
Skinfold thigh1 (mm)	1.2	1.10E-01	0.9	1.70E-01	-1.8	**3.40E-02**	-2.2	**1.30E-02**	-1.7	**4.10E-02**	-0.6	2.90E-01
Skinfold thigh2 (mm)	1.2	1.10E-01	1	1.50E-01	-1.7	**4.40E-02**	-2.1	**1.80E-02**	-1.9	**2.70E-02**	-0.5	3.00E-01
Skinfold thigh3 (mm)	1	1.60E-01	1.1	1.40E-01	-1.8	**3.60E-02**	-2.3	**1.00E-02**	-2	**2.00E-02**	-0.4	3.40E-01

Mean skinfold thigh (mm)	1.2	1.10E-01	1	1.60E-01	-1.8	**3.50E-02**	-2.3	**1.20E-02**	-1.9	**3.20E-02**	-0.5	2.90E-01
Body fat percentage bioimpedance	-0.8	2.20E-01	-1.2	1.20E-01	-1.1	1.30E-01	-1.2	1.10E-01	-0.5	3.10E-01	-0.4	3.60E-01
Body fat (%)	-0.2	4.00E-01	0.8	2.00E-01	-2.6	**5.10E-03**	-2.5	**6.20E-03**	-0.9	1.80E-01	-1.5	7.10E-02
Supine EP (pg/ml)	0.4	3.50E-01	-1.2	1.10E-01	1.6	5.90E-02	1.1	1.40E-01	-0.9	1.80E-01	2.3	**1.20E-02**
Standing EP (pg/ml)	-1	1.60E-01	0.3	3.90E-01	2.5	**6.50E-03**	2.2	**1.40E-02**	-0.3	3.70E-01	2.5	**6.10E-03**
Response EP (pg/ml)	-1.4	8.60E-02	1.1	1.40E-01	1.4	8.50E-02	1.4	7.80E-02	0.3	3.90E-01	0.9	1.70E-01
Supine NE (pg/ml)	0.1	4.70E-01	-0.5	3.10E-01	0.4	3.60E-01	0.4	3.60E-01	0	4.90E-01	0.5	3.00E-01
Standing NE (pg/ml)	-0.5	3.20E-01	-0.1	4.70E-01	1.2	1.20E-01	0.8	2.10E-01	-0.5	3.20E-01	1.2	1.10E-01
Response NE (pg/ml)	-0.4	3.30E-01	0.4	3.30E-01	0.9	1.70E-01	0.7	2.50E-01	-0.6	2.90E-01	0.9	1.80E-01
DBP response to mental stress (mmHg)	1.1	1.30E-01	0.6	2.70E-01	0.5	3.00E-01	0.3	3.90E-01	0	4.90E-01	1.4	7.50E-02
SBP response to mental stress (mmHg)	1.1	1.30E-01	-0.9	1.80E-01	-0.9	1.90E-01	-0.3	3.90E-01	1.3	9.90E-02	-1.4	8.40E-02
HR response to mental stress (beast/min)	-1.7	**4.10E-02**	1.4	7.90E-02	-1.7	**4.10E-02**	-1.8	**4.00E-02**	0.5	3.10E-01	-1.2	1.10E-01
Average sitting DBP (mmHg)	1.2	1.20E-01	-1.1	1.40E-01	-2	**2.10E-02**	-1.7	**4.20E-02**	0.9	1.90E-01	-2.4	**8.80E-03**
Average sitting SBP (mmHg)	0.5	3.20E-01	-1.3	9.10E-02	-3.4	**4.00E-04**	-3.6	**1.40E-04**	-0.5	2.90E-01	-2.3	**1.10E-02**
Average sitting HR (beast/min)	0.6	2.70E-01	1.5	6.60E-02	0.5	3.10E-01	0.4	3.40E-01	-0.1	4.60E-01	0.5	3.10E-01
Mean ambulatory DBP (mmHg)	-0.1	4.60E-01	-1.1	1.30E-01	-2.1	**1.60E-02**	-2	**2.20E-02**	-0.1	4.70E-01	-1.6	**5.00E-02**

Mean ambulatory SBP (mmHg)	-0.2	4.30E-01	-0.8	2.20E-01	-1.3	9.90E-02	-1	1.50E-01	0.2	4.30E-01	-1.5	7.10E-02
Mean ambulatory HR (mmHg)	-0.3	3.90E-01	2.3	**1.20E-02**	-0.4	3.60E-01	-0.6	2.70E-01	-0.2	4.10E-01	0	5.00E-01
Sleep DBP (mmHg)	-0.8	2.20E-01	-1	1.50E-01	-0.6	2.80E-01	-0.7	2.50E-01	-0.3	3.80E-01	0.5	3.00E-01
Sleep SBP (mmHg)	-0.7	2.50E-01	-1.7	**4.10E-02**	0.1	4.60E-01	0	4.80E-01	0	4.90E-01	0.3	3.90E-01
Sleep HR (beast/min)	1.3	1.00E-01	1.9	**2.90E-02**	0.2	4.10E-01	0.3	3.70E-01	0	4.90E-01	0.1	4.60E-01
Sleep MAP (mmHg)	-0.8	2.10E-01	-1.5	7.30E-02	-0.2	4.20E-01	-0.3	3.90E-01	-0.2	4.10E-01	0.5	3.20E-01
Sleep PP (mmHg)	-0.5	3.10E-01	-1.5	6.10E-02	0.8	2.00E-01	0.6	2.60E-01	0	4.80E-01	0.3	4.00E-01
Wake DBP (mmHg)	-0.8	2.00E-01	-0.8	2.10E-01	-0.3	3.70E-01	-0.6	2.60E-01	-0.5	3.20E-01	0	4.90E-01
Wake SBP (mmHg)	-0.8	2.20E-01	0	4.80E-01	0.6	2.60E-01	0.3	3.80E-01	-0.1	4.50E-01	0.7	2.30E-01
Wake HR (beast/min)	0.8	2.00E-01	1.6	5.10E-02	1	1.50E-01	1.7	**4.50E-02**	0.5	3.10E-01	0.1	4.50E-01
Wake MAP (mmHg)	-1	1.50E-01	-0.6	2.80E-01	0.1	4.60E-01	-0.2	4.10E-01	-0.3	3.80E-01	0.3	3.80E-01
Wake PP (mmHg)	0	4.80E-01	0.5	3.20E-01	1.1	1.30E-01	0.9	1.90E-01	0.1	4.70E-01	1	1.60E-01
Overall PP (mmHg)	-0.1	4.50E-01	-0.4	3.50E-01	0.6	2.90E-01	0.7	2.40E-01	0.6	2.80E-01	-0.4	3.60E-01

Table 4. Heritability (H2r) estimates of studied traits by sex[1] and hypertension status[2] and in general[3]

Trait	Females			Males			Normotensives			Hypertensives			All		
	N	SE	H2r	N	SE	H2r	N	SE	H2r	N	SE	H2r	N	SE	H2r
Alcohol users vs. non users	377	0.3	0.85	321	0.2	0.63	326	0.2	0.51	372	0.2	0.55	698	0.2	0.59
Current vs. non tobacco users	377	0.2	0.39	320	0.3	0.62	323	0.2	0.26	374	0.2	0.45	697	0.1	0.39
Current vs. never tobacco users	276	0.2	0.64	196	0.3	0.5	239	0.2	0.51	233	0.3	0.66	472	0.2	0.57
Current vs. former tobacco users	187	0.3	0.23	220	0.3	0.97	191	0.3	0.18	216	0.3	0.62	407	0.2	0.46
Former vs. current and never tobacco users	377	1.1	0.31	320	0.2	0.74	323	0.2	0.28	374	0.2	0.34	697	0.1	0.4
Former vs. never tobacco users	291	0.2	0.56	224	0.4	0.81	216	0.3	0.71	299	0.3	0.43	515	0.1	0.61
Never vs. ever tobacco users	377	0.2	0.58	320	0.2	0.35	323	0.2	0.56	374	0.2	0.36	697	0.1	0.51
Coffee users vs. non users	371	0.2	0.61	308	0.3	0.37	321	0.2	0.66	358	0.2	0.2	679	0.1	0.45
BMI (kg/m2)	429	0.1	0.41	363	0.1	0.22	337	0.1	0.4	455	0.1	0.36	792	0.1	0.34
Waist Hip ratio	422	0.1	0.29	363	0.1	0.34	332	0.1	0.23	453	0.1	0.44	785	0.1	0.32

[1] Data were adjusted for covariate age.
[2] Data were adjusted for covariates, age and sex.
[3] Data were adjusted for covariates, age and sex.

Waist circumference (cm)	423	0.1	0.22	364	0.1	0.31	333	0.1	0.26	454	0.1	0.34	787	0.1	0.29
Hip circumference (cm)	422	0.1	0.28	363	0.1	0.41	332	0.1	0.36	453	0.1	0.37	785	0.1	0.33
Hip Thigh Proximal ratio	409	0.1	0.32	351	0.1	0.24	329	0.1	0.31	431	0.1	0.25	760	0.1	0.24
Thigh proximal circumference (cm)	409	0.1	0.42	353	0.1	0.41	330	0.1	0.42	432	0.1	0.42	762	0.1	0.38
Thigh mid circumference (cm)	412	0.1	0.38	354	0.2	0.45	330	0.1	0.35	436	0.1	0.36	766	0.1	0.33
Thigh distal circumference (cm)	410	0.1	0.42	353	0.1	0.35	330	0.1	0.53	433	0.1	0.36	763	0.1	0.38
Skinfold bicep1 (mm)	362	0.1	0.38	334	0.1	0.65	314	0.1	0.4	382	0.1	0.6	696	0.1	0.46
Skinfold bicep2 (mm)	362	0.1	0.41	334	0.1	0.67	314	0.1	0.41	382	0.1	0.63	696	0.1	0.48
Skinfold bicep3 (mm)	361	0.1	0.43	334	0.1	0.69	314	0.1	0.39	381	0.1	0.64	695	0.1	0.47
Mean skinfold biceps (mm)	362	0.1	0.4	334	0.1	0.67	314	0.1	0.4	382	0.1	0.62	696	0.1	0.47
Skinfold triceps1 (mm)	363	0.1	0.39	334	0.1	0.45	315	0.1	0.26	382	0.1	0.52	697	0.1	0.35
Skinfold triceps2 (mm)	363	0.1	0.37	333	0.1	0.44	315	0.1	0.27	381	0.1	0.48	696	0.1	0.33
Skinfold triceps3 (mm)	362	0.1	0.38	333	0.1	0.42	315	0.1	0.27	380	0.1	0.46	695	0.1	0.33
Mean skinfold triceps (mm)	363	0.1	0.38	334	0.1	0.47	315	0.1	0.26	382	0.1	0.52	697	0.1	0.35
Skinfold subscapular1 (mm)	362	0.1	0.52	331	0.1	0.37	315	0.1	0.37	378	0.1	0.51	693	0.1	0.41

Skinfold subscapular2 (mm)	362	0.1	0.52	331	0.1	0.35	315	0.1	0.36	378	0.1	0.5	693	0.1	0.4
Skinfold subscapular3 (mm)	362	0.1	0.48	331	0.1	0.35	315	0.1	0.36	378	0.1	0.47	693	0.1	0.39
Mean skinfold subscapular (mm)	362	0.1	0.5	331	0.1	0.36	315	0.1	0.36	378	0.1	0.49	693	0.1	0.4
Skinfold suprailiac1 (mm)	360	0.1	0.3	330	0.1	0.35	312	0.1	0.17	378	0.1	0.51	690	0.1	0.3
Skinfold suprailiac2 (mm)	360	0.1	0.31	330	0.1	0.31	312	0.1	0.17	378	0.1	0.52	690	0.1	0.3
Skinfold suprailiac3 (mm)	360	0.1	0.31	330	0.1	0.32	312	0.1	0.17	378	0.1	0.49	690	0.1	0.29
Mean skinfold suprailiac (mm)	360	0.1	0.3	330	0.1	0.32	312	0.1	0.17	378	0.1	0.51	690	0.1	0.29
Skinfold thigh1 (mm)	336	0.1	0.13	324	0.1	0.39	294	0.1	0.28	366	0.1	0.41	660	0.1	0.26
Skinfold thigh2 (mm)	335	0.1	0.08	324	0.1	0.4	293	0.1	0.23	366	0.1	0.4	659	0.1	0.23
Skinfold thigh3 (mm)	335	0.1	0.1	323	0.1	0.4	292	0.1	0.21	366	0.1	0.41	658	0.1	0.24
Mean skinfold thigh (mm)	336	0.1	0.12	324	0.1	0.4	294	0.1	0.26	366	0.1	0.42	660	0.1	0.26
Body fat percentage bioimpedance	314	0.1	0.19	286	0.2	0.58	265	0.2	0.24	335	0.1	0.41	600	0.1	0.32
Body fat (%)	359	0.1	0.33	328	0.1	0.13	311	0.1	0.24	376	0.1	0.34	687	0.1	0.24
Supine EP (pg/ml)	106	0.3	0.33	106	0.3	0.49	123	0.3	0.52	89	0.3	0.72	212	0.2	0.39
Standing EP (pg/ml)	105	0.4	0.35	106	0.3	0.39	122			89	0.3	0.67	211	0.2	0.28

Response EP (pg/ml)	94	0.3	0.23	97	0.3	0.09	117	0.2	0.15	74			191	0.2	0.13
Supine NE (pg/ml)	121	0.3	0.2	117	0.3	0.46	130	0.3	0.05	108	0.3	0.57	238	0.2	0.39
Standing NE (pg/ml)	121	0.2	0.17	116	0.3	0.06	128			109	0.3	0.27	237	0.1	0.18
Response NE (pg/ml)	121	0.2	0.11	115			128			108	0.3	0.07	236	0.1	0.04
DBP response to mental stress (mmHg)	134			135	0.2	0.29	141			128	0.2	0.17	269	0.1	0.11
SBP response to mental stress (mmHg)	133	0.2	0.19	135	0.2	0.17	141			127			268	0.1	0.11
HR response to mental stress (beast/min)	135		0	135	0.3	0.18	142	0.2	0.16	128			270	0.1	0.1
Average sitting DBP (mmHg)	134	0.2	0.53	133	0.2	0.25	141	0.2	0.69	126	0.3	0.33	267	0.1	0.41
Average sitting SBP (mmHg)	134	0.2	0.54	133	0.3	0.66	141	0.2	0.48	126	0.2	0.1	267	0.1	0.42
Average sitting HR (beast/min)	134	0.2	0.67	131	0.2	0.76	141	0.2	0.87	124	0.3	0.34	265	0.1	0.58
Mean ambulatory DBP (mmHg)	104	0.3	0.47	99	0.3	0.7	109	0.3	0.6	94	0.3	0.27	203	0.2	0.46
Mean ambulatory SBP (mmHg)	104	0.2	0.49	99	0.3	0.19	109			94			203	0.1	0.26
Mean ambulatory HR (mmHg)	104	0.3	0.67	99	0.3	0.81	109	0.3	0.99	94			203	0.2	0.64
Sleep DBP (mmHg)	124	0.2	0.07	124	0.2	0.47	135	0.3	0.18	113	0.2	0.47	248	0.2	0.37
Sleep SBP (mmHg)	124	0.3	0.39	124	0.2	0.32	135	0.3	0.14	113	0.2	0.23	248	0.1	0.31

Sleep HR (beast/min)	123	0.3	0.33	123	0.2	0.72	134	0.2	0.74	112	0.3	0.13	246	0.2	0.54
Sleep MAP (mmHg)	124	0.3	0.23	124	0.2	0.47	135	0.3	0.32	113	0.2	0.35	248	0.2	0.4
Sleep PP (mmHg)	124	0.3	0.33	124	0.2	0.16	135			113	0.2	0.25	248	0.1	0.14
Wake DBP (mmHg)	122			125	0.2	0.52	135			112	0.2	0.27	247	0.1	0.24
Wake SBP (mmHg)	122	0.3	0.55	125	0.2	0.35	135	0.3	0.27	112	0.2	0.3	247	0.1	0.39
Wake HR (beast/min)	122	0.3	0.59	125	0.2	0.75	135	0.3	0.64	112	0.3	0.66	247	0.2	0.63
Wake MAP (mmHg)	122	0.2	0.11	125	0.2	0.47	135	0.3	0.09	112	0.2	0.24	247	0.1	0.31
Wake PP (mmHg)	122	0.2	0.68	125	0.2	0.25	135	0.2	0.36	112	0.3	0.46	247	0.1	0.28
Overall PP (mmHg)	104	0.3	0.41	99	0.2	0.1	109	0.3	0.02	94	0.3	0.26	203	0.1	0.24

Table 5. Candidate loci driven by joint linkage and family-based association analysis

Chr	Trait	Linkage			Association			Corrected P
		LOD Score	cM	Number of SNPs[1]	SNP	P	Gene	
1	Alcohol use	2	111-119	116	rs1281590	1.1E-04	LPHN2;TTLL7	1.2E-02
				116	rs925076	2.0E-04	COL24A1;ODF2L	2.3E-02
2	HR response to mental stress	2.4	144-148	114	rs10496870	3.7E-04	LRP1B	4.2E-02
3	Average sitting HR	1.95	154-165	95	rs1492090	3.7E-04	AGTR1	3.5E-02
4	Current tobacco use	1.75	98-106	150	rs4447825	3.0E-06	GRID2	4.5E-04
4	Sleep MAP	2.2	165-166	36	rs954709	1.4E-04	TLL1	4.9E-03
5	Coffee use	2.56	36-59	246	rs563972	1.8E-04	DAB2;PTGER4	4.4E-02
7	NE standing	2.1	140-147	50	rs834767	3.7E-05	CHRM2;MTPN	1.9E-03

[1] Numbers of SNPs that are within 1 LOD-drop interval around the linkage peak.

8	Never tobacco use	2	28-38	139	rs3739401	1.5E-04	PCM1	2.0E-02
12	HR response to mental stress	2.8	46-52	91	rs4414322	3.1E-04	ITPR2	2.8E-02
				91	rs708156	3.2E-04	ITPR2	2.9E-02
13	Sleep MAP	2	39-46	179	rs927544	1.5E-04	HTR2A	2.7E-02
13	DBP response to mental stress	2.9	45.5-48	76	rs2275664	4.4E-04	PRR20	3.3E-02
18	Skinfold biceps	2.02	99-108	152	rs10514053	3.1E-05	CBLN2;NETO1	4.7E-03
					rs10514055	2.2E-04	NETO1	3.4E-02

Table 6. Shared candidate SNPs among the studied traits driven from family based association analysis

Trait	Chr	SNP	P	Gene	Combined P
Current vs. former tobacco users	1	rs951908	8.8E-04	UBR4	7.6E-08
Body fat	1	rs1009806	4.2E-06	UBR4	
Skinfold triceps	1	rs320035	4.9E-04	AGBL4	
Never vs. ever tobacco users	1	rs679783	3.0E-05	AGBL4	
Waist circumference	1	rs679783	7.4E-05	AGBL4	4.6E-08
Never vs. ever tobacco users	1	rs354167	1.6E-04	AGBL4	
Never vs. ever tobacco users	1	rs1112687	1.4E-04	AGBL4	
SBP response to mental stress	1	rs1404072	4.0E-04	ELTD1	
BMI	1	rs1404072	4.5E-04	ELTD1	2.3E-10
Sleep SBP	1	rs10493646	3.6E-04	ELTD1	
Alcohol users vs. non users	1	rs10493646	6.6E-04	ELTD1	
Skinfold biceps	1	rs1340128	1.9E-04	KCNH1	7.7E-07
DBP response to mental stress	1	rs1112269	2.2E-04	KCNH1	
SBP response to mental stress	2	rs1522984	7.3E-04	PRKCE	
Wake DBP	2	rs872288	9.3E-04	PRKCE	1.1E-07
Standing NE	2	rs951012	6.0E-04	PRKCE	

Hip circumference	2	rs974736	5.3E-04	CTNNA2	
Hip circumference	2	rs2916484	2.4E-04	CTNNA2	
SBP response to mental stress	2	rs408144	4.7E-04	CTNNA2	3.8E-10
Former vs. never tobacco users	2	rs10496238	8.0E-06	CTNNA2	
Response NE	2	rs1385775	6.7E-04	LRP1B	
Standing NE	2	rs10496864	7.4E-04	LRP1B	
HR response to mental stress	2	rs4133302	6.3E-04	LRP1B	2.5E-09
HR response to mental stress	2	rs10496870	4.8E-05	LRP1B	
Mean ambulatory DBP	2	rs1521102	2.1E-04	LRP1B	
Average sitting SBP	2	rs10497170	1.0E-05	NR4A2	3.1E-08
SBP response to mental stress	2	rs10497170	1.5E-04	NR4A2	
Hip Thigh Proximal ratio	2	rs10490326	1.1E-04	CPS1	
Former vs. never tobacco users	2	rs10490326	7.1E-04	CPS1	1.3E-06
Standing EP	3	rs1516391	5.0E-04	CNTN4	
Response EP	3	rs1400205	9.6E-04	CNTN4	
Never vs. ever tobacco users	3	rs339287	3.9E-04	CNTN4	4.0E-07
Never vs. ever tobacco users	3	rs339286	4.3E-05	CNTN4	
Mean ambulatory HR	3	rs10510314	3.9E-05	EDEM1	
HR response to mental stress	3	rs10510314	1.2E-04	EDEM1	9.4E-08

Hip Thigh Proximal ratio	3	rs3905330	1.7E-04	DAG1	7.5E-07
DBP response to mental stress	3	rs3905330	2.5E-04	DAG1	
Skinfold subscapular	3	rs1882898	3.1E-04	FHIT	
Skinfold subscapular	3	rs1882899	1.3E-04	FHIT	
Skinfold subscapular	3	rs963685	4.5E-05	FHIT	6.0E-07
Mean ambulatory DBP	3	rs953480	7.3E-04	FHIT	
Mean ambulatory DBP	3	rs953479	7.3E-04	FHIT	
Thigh proximal circumference	3	rs6767186	3.1E-05	PHLDB2	5.5E-07
Average sitting DBP	3	rs6767186	9.6E-04	PHLDB2	
Waist circumference	4	rs6532079	2.6E-04	FAM13A1	1.2E-06
HR response to mental stress	4	rs6532079	2.6E-04	FAM13A1	
DBP response to mental stress	4	rs720327	4.7E-04	FAM190A	
Response EP	4	rs10516878	6.6E-04	FAM190A	7.1E-08
Thigh mid circumference	4	rs1919224	8.7E-04	FAM190A	
Skinfold thigh	4	rs7663835	9.8E-04	GRID2	
Current vs. never tobacco users	4	rs1369169	4.2E-05	GRID2	5.2E-08
Current vs. former tobacco users	4	rs2085364	2.6E-06	GRID2	
Wake SBP	4	rs1533875	1.0E-04	ODZ3	4.2E-07

Response NE	4	rs1533875	2.2E-04	ODZ3	
Never vs. ever tobacco users	5	rs410112	2.1E-05	MYO10;FAM134B	
Waist circumference	5	rs410112	7.4E-04	MYO10;FAM134B	
Never vs. ever tobacco users	5	rs2434517	1.5E-04	MYO10;FAM134B	
Waist circumference	5	rs2434517	9.6E-04	MYO10;FAM134B	2.7E-07
Never vs. ever tobacco users	5	rs588367	2.7E-04	MYO10;FAM134B	
Waist circumference	5	rs588367	7.2E-04	MYO10;FAM134B	
Never vs. ever tobacco users	5	rs876095	5.3E-05	MYO10;FAM134B	
Waist circumference	5	rs876095	6.9E-04	MYO10;FAM134B	
Coffee users vs. non users	5	rs32441	1.3E-04	ST8SIA4;FAM174A	1.1E-06
Sleep PP	5	rs32441	4.9E-04	ST8SIA4;FAM174A	
Body fat percentage bioimpedance	5	rs10515313	1.6E-04	ST8SIA4;SLCO4C1	1.2E-06
Mean ambulatory HR	5	rs10515313	4.1E-04	ST8SIA4;SLCO4C1	
Coffee users vs. non users	5	rs960452	7.3E-05	CAMK4	1.1E-06
Supine EP	5	rs9326835	8.8E-04	CAMK4	
Coffee users vs. non users	5	rs4835804	6.1E-05	SNX2;SNCAIP	7.5E-07
Standing NE	5	rs4835804	6.9E-04	SNX2;SNCAIP	
Thigh mid circumference	5	rs6881950	1.1E-04	ODZ2	7.9E-07
SBP response to mental stress	5	rs1472356	4.2E-04	ODZ2	

Average sitting DBP	6	rs1364557	6.3E-05	FARS2	7.5E-07
Thigh distal circumference	6	rs10484311	6.6E-04	FARS2	
Coffee users vs. non users	6	rs9321180	8.9E-05	ARHGAP18;C6orf191	5.3E-07
Former vs. never tobacco users	6	rs9321180	3.3E-04	ARHGAP18;C6orf191	
Never vs. ever tobacco users	7	rs7800027	2.5E-05	DGKB	4.2E-07
Skinfold subscapular	7	rs7810871	9.0E-04	DGKB	
Standing EP	8	rs1499682	9.0E-04	CSMD1	1.5E-12
Standing EP	8	rs2100119	5.7E-04	CSMD1	
Coffee users vs. non users	8	rs810437	4.7E-04	CSMD1	
Never vs. ever tobacco users	8	rs810437	4.9E-04	CSMD1	
Wake DBP	8	rs1350307	2.2E-04	CSMD1	
Body fat percentage bioimpedance	8	rs1350307	5.1E-04	CSMD1	
Wake DBP	8	rs1673243	2.4E-04	CSMD1	
Body fat percentage bioimpedance	8	rs1673243	5.9E-04	CSMD1	
Waist Hip ratio	8	rs10503296	6.5E-04	CSMD1	
Wake HR	8	rs10503484	7.0E-04	SGCZ	3.0E-10
Current vs. never tobacco users	8	rs1381418	7.8E-04	SGCZ	

Hip Thigh Proximal ratio	8	rs2035141	1.3E-06	SGCZ	
Supine EP	8	rs2299594	8.9E-04	PCM1	
Coffee users vs. non users	8	rs2299594	9.9E-04	PCM1	
Never vs. ever tobacco users	8	rs208756	3.2E-04	PCM1	
Never vs. ever tobacco users	8	rs3739401	1.5E-04	PCM1	2.3E-10
Skinfold suprailiac	8	rs3739401	3.4E-04	PCM1	
Hip circumference	8	rs10503607	4.1E-04	PCM1	
Never vs. ever tobacco users	8	rs10503607	8.4E-04	PCM1	
Thigh mid circumference	8	rs10503649	5.0E-04	CSGALNACT1	
Current vs. never tobacco users	8	rs10503658	1.4E-04	CSGALNACT1	1.2E-06
Skinfold biceps	8	rs421501	5.7E-05	PXMP3;PKIA	6.0E-07
Mean ambulatory DBP	8	rs421501	5.8E-04	PXMP3;PKIA	
Sleep PP	9	rs1434276	3.4E-04	PTPRD	
Skinfold thigh	9	rs867980	2.0E-04	PTPRD	3.0E-12
Supine EP	9	rs664921	4.1E-05	PTPRD	
Never vs. ever tobacco users	9	rs725262	1.3E-04	PTPRD	
Sleep HR	9	rs2378665	3.0E-05	SLC28A3;RMI1	2.3E-07
Average sitting DBP	9	rs2378665	3.9E-04	SLC28A3;RMI1	
Hip Thigh Proximal ratio	10	rs4933268	7.5E-04	NRG3	7.0E-08

Hip Thigh Proximal ratio	10	rs7069222	9.8E-04	NRG3	
Former vs. never tobacco users	10	rs1336274	4.6E-06	NRG3	
Hip Thigh Proximal ratio	11	rs3905300	7.9E-04	ODZ4	
Response NE	11	rs10501444	3.2E-05	ODZ4	2.9E-09
SBP response to mental stress	11	rs10501444	3.2E-04	ODZ4	
Thigh distal circumference	11	rs4143314	9.1E-04	DLG2	
Wake MAP	11	rs1384753	2.7E-05	DLG2	
Hip circumference	11	rs1384754	4.4E-04	DLG2	
DBP response to mental stress	11	rs10501570	1.6E-05	DLG2	7.5E-13
Response EP	11	rs651661	9.5E-04	DLG2	
Skinfold thigh	11	rs473968	9.0E-04	DLG2	
Skinfold thigh	11	rs1213257	2.0E-04	DLG2	
Coffee users vs. non users	11	rs16915547	6.2E-06	CHORDC1	
Former vs. never tobacco users	11	rs16915547	6.4E-04	CHORDC1	8.1E-08
HR response to mental stress	12	rs4414322	3.1E-04	ITPR2	
HR response to mental stress	12	rs708156	1.0E-03	ITPR2	
Supine NE	12	rs10506006	1.4E-04	ITPR2	7.7E-09
Current vs. former tobacco users	12	rs728009	5.3E-04	ITPR2	
Current vs. former tobacco	12	rs10506011	9.0E-04	ITPR2	

users

Mean ambulatory HR	12	rs10492303	4.4E-05	MDM1;RAP1B	5.1E-07
Mean ambulatory DBP	12	rs10492303	6.3E-04	MDM1;RAP1B	
Coffee users vs. non users	12	rs4762559	2.3E-05	ANKS1B	4.5E-08
Never vs. ever tobacco users	12	rs4762559	3.4E-04	ANKS1B	
Coffee users vs. non users	12	rs10507107	4.2E-05	ANKS1B	
Never vs. ever tobacco users	12	rs10507107	9.3E-05	ANKS1B	
Hip circumference	13	rs3803261	4.6E-04	OLFM4	2.0E-12
Waist circumference	13	rs3803260	7.5E-05	OLFM4	
Never vs. ever tobacco users	13	rs3803260	8.2E-04	OLFM4	
Alcohol users vs. non users	13	rs7997432	9.8E-05	OLFM4	
Coffee users vs. non users	13	rs7997432	3.4E-04	OLFM4	
Coffee users vs. non users	13	rs9285197	4.0E-05	OLFM4	
Skinfold biceps	13	rs10507651	8.1E-05	TDRD3	9.2E-07
Sleep MAP	13	rs10507651	6.4E-04	TDRD3	
Coffee users vs. non users	13	rs7981910	5.5E-05	GPC6	1.8E-12
Hip Thigh Proximal ratio	13	rs4412846	7.0E-04	GPC6	
Hip Thigh Proximal ratio	13	rs920956	1.7E-04	GPC6	
Mean ambulatory SBP	13	rs1584147	5.5E-04	GPC6	
Hip Thigh Proximal ratio	13	rs1933146	1.2E-04	GPC6	

Hip Thigh Proximal ratio	13	rs9284276	1.1E-04	GPC6	
Supine NE	13	rs9284276	8.9E-04	GPC6	
Hip Thigh Proximal ratio	13	rs6650320	4.4E-05	GPC6	
Supine NE	13	rs6650320	1.6E-04	GPC6	
Hip Thigh Proximal ratio	13	rs6650321	6.6E-05	GPC6	
Sleep HR	14	rs1954668	5.7E-04	NOVA1	
Alcohol users vs. non users	14	rs1954673	3.7E-04	NOVA1	5.0E-08
Coffee users vs. non users	14	rs1954673	8.7E-04	NOVA1	
Average sitting HR	15	rs10519074	4.2E-04	RORA	
Skinfold subscapular	15	rs10519076	3.1E-04	RORA	
Skinfold thigh	15	rs10519095	3.6E-04	RORA	2.2E-09
Wake MAP	15	rs726914	9.1E-05	RORA	
Wake MAP	15	rs726913	4.8E-05	RORA	
Skinfold subscapular	16	rs9302844	3.6E-05	A2BP1	
Supine EP	16	rs740676	1.4E-04	A2BP1	9.7E-08
Supine EP	16	rs763650	1.3E-04	A2BP1	
Coffee users vs. non users	17	rs2469828	1.1E-04	TMEM99;KRT12	3.6E-07
Never vs. ever tobacco users	17	rs2469828	1.8E-04	TMEM99;KRT12	
Coffee users vs. non users	18	rs10502334	1.4E-04	L3MBTL4;EPB41L3	9.4E-07
Hip Thigh Proximal ratio	18	rs10502334	3.9E-04	L3MBTL4;EPB41L3	

Coffee users vs. non users	18	rs640128	4.7E-04	PTPRM	2.1E-08
SBP response to mental stress	18	rs4798593	2.1E-06	PTPRM	
SBP response to mental stress	18	rs4890429	6.2E-05	RIT2;SYT4	3.2E-07
BMI	18	rs4890429	2.8E-04	RIT2;SYT4	
Coffee users vs. non users	20	rs6135159	7.0E-05	MACROD2	
Current vs. non tobacco users	20	rs10485518	7.7E-05	MACROD2	
Current vs. former tobacco users	20	rs10485531	5.6E-04	MACROD2	1.1E-07
Current vs. former tobacco users	20	rs2023385	7.5E-04	MACROD2	

Table 7. Hypertension-specific and sex-specific candidate loci driven by joint linkage and family-based association analysis

Status	Chr	Trait	Linkage			Association			Corrected P
			LOD Score	cM	Number of SNPs[1]	SNP	P	Gene	
Hypertensives	1	Body fat percentage bioimpedance	2.8	188-192	113	rs7526653	4.4E-05	DNM3	5.0E-03
					113	rs9286842	3.1E-04	DNM3	3.5E-02
					113	rs2861812	6.4E-05	PAPPA2	7.2E-03
Hypertensives	1	Hip circumference	2.65	184-189	64	rs593479	1.7E-04	PRRX1	1.1E-02
Hypertensives	1	Thigh distal circumference	2.83	166-176	92	rs2841979	1.3E-05	RGS4;C1orf110	1.2E-03
Hypertensives	1	Thigh proximal circumference	2.9	187-191	76	rs593479	8.6E-05	PRRX1	6.5E-03
Hypertensives	3	Hip circumference	2.5	170-172	57	rs1874952	6.1E-04	KCNAB1	3.5E-02
Normotensives	4	Sleep HR	2.3	80-88	126	rs7700235	1.7E-04	ADAMTS3	2.1E-02
					126	rs788910	2.7E-04	ADAMTS3	3.4E-02

[1] Numbers of SNPs that are within 1 LOD-drop interval around the linkage peak.

Hypertensives	5	Skinfold subscapular	3.1	36-40	51	rs10520888	4.4E-04	PRDM9;CDH12	2.3E-02
Normotensives	5	Standing EP	2	120-127	77	rs254150	1.6E-04	COMMD10;SEMA6A	1.2E-02
Hypertensives	6	Hip circumference	3.8	27-29	5	rs4486015	3.3E-04	DTNBP1;MYLIP	1.6E-03
Hypertensives	10	Hip circumference	2.8	59-61	18	rs2065443	1.4E-04	ARHGAP12	2.4E-03
					18	rs1775715	2.2E-04	KIF5B	4.0E-03
Normotensives	11	HR response to mental stress	3.9	18-25	114	rs4757397	2.9E-04	SOX6	3.3E-02
Normotensives	15	Waist Hip ratio	2	49-56	90	rs10519116	9.7E-05	RORA	8.7E-03
Hypertensives	16	Hip Thigh Proximal ratio	3.3	28-36	51	rs2023763	2.3E-04	SYT17;TMC5	1.2E-02
Hypertensives	21	Skinfold biceps	2.46	34-45	104	rs2249248	1.4E-04	DSCAM;BACE2	1.5E-02
Males	1	Body fat percentage bioimpedance	2.02	186-206	320	rs4471302	1.4E-04	DNM3	4.5E-02

Females	13	Mean skinfold suprailiac	2.1	44-45.5	14	rs3803261	2.8E-04	OLFM4	4.0E-03
Females	17	Wake PP	2.03	90-102	101	rs10512596	6.0E-06	CD300LB	6.0E-04
						rs783250	5.3E-05	CD300LB	5.4E-03

Table 8. Hypertension-specific and sex-specific shared candidate SNPs among the studied traits driven from family based association analysis

Status	Trait	Chr	SNP	P	Gene	Combined P
Normotensives	Supine EP	4	rs6855889	5.7E-04	CPE	
Normotensives	Skinfold thigh	4	rs7692208	3.0E-04	CPE	
Normotensives	Alcohol users vs. non users	4	rs10517848	9.6E-04	CPE	2.3E-08
Normotensives	Skinfold thigh	4	rs10517848	9.4E-04	CPE	
Normotensives	Alcohol users vs. non users	4	rs4481204	4.6E-04	CPE	
Normotensives	Supine EP	4	rs1898593	6.8E-04	CPE	
Normotensives	Wake MAP	4	rs4128165	1.7E-04	GLRA3	
Normotensives	Skinfold biceps	4	rs1352055	7.0E-04	GLRA3	9.8E-07
Normotensives	Skinfold biceps	4	rs1385832	3.3E-04	GLRA3	
Normotensives	Overall PP	5	rs4913079	2.4E-04	PFDN1;HBEGF	4.9E-07
Normotensives	Waist circumference	5	rs4913079	1.1E-04	PFDN1;HBEGF	
Normotensives	Waist circumference	7	rs1880610	7.9E-04	SDK1	1.1E-08

Normotensives	Thigh distal circumference	7	rs10499327	1.4E-04	SDK1	
Normotensives	Coffee users vs. non users	7	rs956393	4.0E-04	SDK1	
Normotensives	Coffee users vs. non users	7	rs10485876	3.4E-04	SDK1	
Normotensives	HR response to mental stress	7	rs10485873	7.4E-04	SDK1	
Normotensives	Standing EP	15	rs7166370	3.4E-05	RORA	6.8E-08
Normotensives	Waist Hip ratio	15	rs10519116	9.7E-05	RORA	
Hypertensives	Coffee users vs. non users	5	rs10515427	3.0E-04	CAMK4	
Hypertensives	Coffee users vs. non users	5	rs306079	6.0E-04	CAMK4	
Hypertensives	Skinfold subscapular	5	rs306079	4.2E-04	CAMK4	8.0E-07
Hypertensives	Coffee users vs. non users	5	rs10491334	3.4E-04	CAMK4	
Hypertensives	Coffee users vs. non users	5	rs9326835	1.1E-04	CAMK4	
Hypertensives	Hip circumference	5	rs10515597	3.4E-04	SPINK5	
Hypertensives	DBP response to mental stress	5	rs10515597	1.3E-04	SPINK5	2.1E-07
Hypertensives	Hip circumference	5	rs10515599	8.6E-05	SPINK5	

Hypertensives	DBP response to mental stress	5	rs10515599	3.2E-04	SPINK5	
Hypertensives	Sleep SBP	6	rs6905827	7.0E-04	MAN1A1	6.2E-07
Hypertensives	Never vs. ever tobacco users	6	rs6905827	4.9E-05	MAN1A1	
Hypertensives	Current vs. never tobacco users	6	rs1028571	8.9E-04	NKAIN2	
Hypertensives	Current vs. non tobacco users	6	rs1028572	2.2E-04	NKAIN2	4.7E-08
Hypertensives	Supine EP	6	rs2552085	8.6E-04	NKAIN2	
Hypertensives	Overall PP	6	rs1842129	9.3E-04	NKAIN2	
Hypertensives	Skinfold triceps	8	rs17079530	5.7E-04	CSMD1	1.2E-06
Hypertensives	Sleep PP	8	rs10503263	1.2E-04	CSMD1	
Hypertensives	Skinfold triceps	9	rs10511522	2.9E-04	PTPRD	
Hypertensives	Never vs. ever tobacco users	9	rs768224	1.0E-04	PTPRD	9.3E-09
Hypertensives	Mean ambulatory HR	9	rs7023147	9.6E-04	PTPRD	
Hypertensives	Mean ambulatory HR	10	rs10509265	7.5E-04	CTNNA3	7.4E-07

Hypertensives	Coffee users vs. non users	10	rs2894028	5.5E-05	CTNNA3	
Hypertensives	Wake MAP	11	rs726533	4.1E-04	CNTN5	5.0E-07
Hypertensives	Waist circumference	11	rs726533	6.6E-05	CNTN5	
Hypertensives	Hip circumference	13	rs1927762	9.4E-07	FAM155A	2.5E-09
Hypertensives	Former vs. current and never tobacco users	13	rs3905075	1.1E-04	FAM155A	
Hypertensives	Coffee users vs. non users	14	rs1955639	4.1E-04	C14orf37	1.1E-06
Hypertensives	Thigh proximal circumference	14	rs178493	1.6E-04	C14orf37	
Hypertensives	Hip circumference	18	rs8091206	1.4E-04	ZNF532	1.0E-06
Hypertensives	Former vs. current and never tobacco users	18	rs8091206	4.3E-04	ZNF532	
Females	Never vs. ever tobacco users	3	rs339287	3.9E-05	CNTN4	4.0E-07
Females	Waist Hip ratio	3	rs339286	5.6E-04	CNTN4	
Females	Wake HR	3	rs304039	6.5E-04	ITPR1	1.8E-09

Females	Average sitting DBP	3	rs3804992	3.7E-05	ITPR1	
Females	Skinfold subscapular	3	rs4685834	2.0E-04	ITPR1	
Females	Skinfold subscapular	3	rs9284900	2.5E-04	FHIT	
Females	Average sitting HR	3	rs213342	8.9E-04	FHIT	
Females	Waist circumference	3	rs1882898	2.4E-04	FHIT	1.9E-08
Females	Waist circumference	3	rs1882899	9.1E-05	FHIT	
Females	Waist circumference	3	rs963685	8.1E-05	FHIT	
Females	Response NE	3	rs7631068	8.8E-04	FHIT	
Females	Coffee users vs. non users	3	rs2089352	3.1E-04	CADPS	3.7E-07
Females	Mean ambulatory HR	3	rs523320	6.4E-05	CADPS	
Females	SBP response to mental stress	3	rs583911	5.5E-05	IL12A	6.7E-07
Females	Current vs. never tobacco users	3	rs583911	6.7E-04	IL12A	
Females	Alcohol users vs. non users	4	rs10519407	5.2E-04	PCDH18	6.8E-11

Females	Alcohol users vs. non users	4	rs1011403	1.1E-04	PCDH18	
Females	Alcohol users vs. non users	4	rs1517945	1.1E-04	PCDH18	
Females	Alcohol users vs. non users	4	rs1517946	1.1E-04	PCDH18	
Females	HR response to mental stress	4	rs633403	2.9E-04	PCDH18	
Females	HR response to mental stress	4	rs536520	3.6E-04	PCDH18	
Females	Thigh mid circumference	4	rs534612	4.5E-04	PCDH18	
Females	Alcohol users vs. non users	4	rs10519412	1.4E-04	PCDH18	
Females	Response NE	4	rs1816176	7.5E-04	PCDH18	
Females	Thigh distal circumference	4	rs1816176	8.6E-04	PCDH18	
Females	Coffee users vs. non users	5	rs1392452	6.8E-05	CDH6	2.3E-07
Females	Waist Hip ratio	5	rs1392452	1.7E-04	CDH6	
Females	Skinfold thigh	6	rs9294628	9.3E-04	EYS	
Females	Waist Hip ratio	6	rs10485054	7.1E-05	EYS	3.8E-09
Females	Current vs. former tobacco users	6	rs10485054	3.6E-04	EYS	

Females	Current vs. never tobacco users	6	rs974110	2.6E-04	EYS	
Females	Current vs. former tobacco users	6	rs354362	8.1E-04	EYS	
Females	Mean ambulatory SBP	6	rs10484935	5.9E-04	EYS	
Females	BMI	6	rs9294806	1.4E-04	BAI3	
Females	Waist Hip ratio	6	rs10485434	2.0E-04	BAI3	9.4E-07
Females	Never vs. ever tobacco users	6	rs10485433	3.8E-04	BAI3	
Females	Mean ambulatory SBP	7	rs4385381	8.7E-04	INHBA	
Females	Thigh distal circumference	7	rs4385381	7.2E-04	INHBA	
Females	BMI	7	rs1014106	7.8E-04	INHBA	9.1E-08
Females	Former vs. never tobacco users	7	rs1014106	5.6E-04	INHBA	
Females	Waist Hip ratio	12	rs816203	1.9E-04	KSR2	
Females	Response NE	12	rs1878419	2.9E-04	KSR2	9.6E-07
Females	BMI	13	rs10507433	4.3E-05	DCLK1	8.1E-08

Females	Current vs. non tobacco users	13	rs7334245	9.3E-05	DCLK1	
Females	Current vs. never tobacco users	13	rs9315383	8.1E-04	DCLK1	
Females	Coffee users vs. non users	14	rs766146	2.3E-04	STRN3	
Females	Waist Hip ratio	14	rs766146	9.7E-05	STRN3	
Females	Coffee users vs. non users	14	rs9322866	6.2E-05	STRN3	4.1E-10
Females	Waist Hip ratio	14	rs9322866	1.8E-05	STRN3	
Females	DBP response to mental stress	14	rs9322866	9.0E-04	STRN3	
Females	Coffee users vs. non users	14	rs1179966	7.2E-05	SLC25A21	1.2E-06
Females	Skinfold subscapular	14	rs1956425	9.5E-04	SLC25A21	
Females	Waist circumference	14	rs10483492	8.5E-04	C14orf25	
Females	Wake PP	14	rs728087	9.0E-05	C14orf25	1.3E-06
Females	Wake SBP	14	rs6571820	5.5E-04	C14orf25	
Females	Coffee users vs. non users	14	rs7150974	5.3E-04	NRXN3	4.3E-07

Females	Wake HR	14	rs7141526	4.4E-05	NRXN3	
Males	Skinfold thigh	3	rs1873038	5.4E-04	NLGN1	
Males	Sleep HR	3	rs725802	8.1E-04	NLGN1	2.5E-08
Males	Sleep HR	3	rs725800	7.6E-04	NLGN1	
Males	DBP response to mental stress	3	rs4894664	2.0E-04	NLGN1	
Males	Sleep HR	7	rs847936	5.5E-05	VWDE;SCIN	8.8E-07
Males	Never vs. ever tobacco users	7	rs847936	9.0E-04	VWDE;SCIN	
Males	Waist Hip ratio	8	rs10504932	4.4E-04	CDH17;PDP1	2.7E-07
Males	Former vs. current and never tobacco users	8	rs10504932	3.3E-05	CDH17;PDP1	
Males	Coffee users vs. non users	8	rs10505508	1.7E-04	PVT1	1.1E-06
Males	Former vs. current and never tobacco users	8	rs10505508	3.6E-04	PVT1	
Males	Sleep HR	8	rs10505551	7.7E-04	ADCY8;ASAP1	8.6E-07
Males	Skinfold subscapular	8	rs10505551	6.3E-05	ADCY8;ASAP1	

Males	Waist circumference	12	rs958644	8.2E-04	PTPRR	
Males	Mean skinfold subscapular	12	rs10506604	6.1E-05	PTPRR	6.0E-07
Males	Sleep DBP	12	rs2458439	5.4E-04	PTPRR	
Males	Sleep DBP	12	rs2458437	7.1E-04	PTPRR	
Males	Alcohol users vs. non users	12	rs10506912	3.2E-04	LRRIQ1	
Males	Coffee users vs. non users	12	rs10506912	1.0E-04	LRRIQ1	
Males	Coffee users vs. non users	12	rs2404772	8.8E-04	LRRIQ1	5.9E-07
Males	Alcohol users vs. non users	12	rs1159869	5.8E-04	LRRIQ1	
Males	Coffee users vs. non users	12	rs1159869	2.1E-04	LRRIQ1	
Males	Current vs. non tobacco users	13	rs596425	1.4E-04	SGCG	
Males	Current vs. non tobacco users	13	rs512444	3.1E-04	SGCG	7.7E-07
Males	Standing NE	13	rs1359978	3.1E-04	SGCG	
Males	Waist circumference	13	rs10507737	4.0E-05	PCDH9	6.6E-07

Males	HR response to mental stress	13	rs10507737	9.1E-04	PCDH9
Males	Waist Hip ratio	13	rs10507739	5.5E-04	PCDH9

Table 9. Comparing the gene expression profiles among the shared genes driven from general analysis, sex-specific and hypertension-specific analysis

General				Sex-specific				Hypertension-specific			
Tissue	N	Mean	SE	Tissue	N	Mean	SE	Tissue	N	Mean	SE
Temporal lobe	60	0.33	0.1	Cerebellum	27	0.31	0.2	Cerebellum	16	0.62	0.3
Occipital lobe	60	0.32	0.1	Accumbens	27	0.31	0.1	Temporal lobe	16	0.56	0.3
Accumbens	60	0.32	0.1	Occipital lobe	27	0.30	0.2	Amygdala	16	0.52	0.2
Frontal lobe	60	0.32	0.1	Parietal lobe	27	0.30	0.2	Accumbens	16	0.52	0.3
Parietal lobe	60	0.32	0.1	Frontal lobe	27	0.29	0.1	Parietal lobe	16	0.52	0.3
Amygdala	60	0.29	0.1	Putamen	27	0.29	0.1	Cerebral cortex	16	0.50	0.3
Cerebral cortex	60	0.29	0.1	Temporal lobe	27	0.27	0.1	Putamen	16	0.50	0.3
Dorsal root ganglia	60	0.28	0.1	Amygdala	27	0.27	0.1	Frontal lobe	16	0.50	0.2
Putamen	60	0.27	0.1	Cerebral cortex	27	0.27	0.1	Occipital lobe	16	0.49	0.3
Hippocampus	60	0.26	0.1	Hippocampus	27	0.25	0.1	Hippocampus	16	0.49	0.2
Thalamus	60	0.24	0.1	Spinal cord	27	0.23	0.2	Medulla	16	0.44	0.3
Hypothalamus	60	0.23	0.1	Ventral tegmental area	27	0.22	0.1	Nodose nucleus	16	0.43	0.3

Cerebellum	60	0.23	0.1	Vestibular nuclei sup	27	0.21	0.1	Corpus callosum	16	0.43	0.3
Vestibular nuclei sup	60	0.22	0.1	Thalamus	27	0.21	0.1	Spinal cord	16	0.41	0.3
Trigeminal ganglia	60	0.20	0.1	Medulla	27	0.20	0.1	Midbrain	16	0.39	0.3
Medulla	60	0.19	0.1	Substantia nigra	27	0.16	0.1	Substantia nigra	16	0.38	0.2
Ventral tegmental area	60	0.16	0.1	Midbrain	27	0.16	0.1	Hypothalamus	16	0.38	0.2
Spinal cord	60	0.16	0.1	Heart atrium	27	0.11	0.2	Thalamus	16	0.38	0.2
Midbrain	60	0.15	0.1	Hypothalamus	27	0.10	0.1	Vestibular nuclei sup	16	0.38	0.2
Substantia nigra	60	0.10	0.1	Saphenous vein	27	0.10	0.1	Subthalamic nucleus	16	0.37	0.3
Heart atrium	60	0.09	0.1	Colon cecum	27	0.09	0.1	Ventral tegmental area	16	0.36	0.2
Corpus callosum	60	0.05	0.1	Dorsal root ganglia	27	0.07	0.1	Cervix	16	0.13	0.2
Pituitary gland	60	0.04	0.1	Heart ventricle	27	0.07	0.2	Vagina	16	0.11	0.2
Vagina	60	0.03	0.1	Subthalamic nucleus	27	0.06	0.1	Dorsal root ganglia	16	0.05	0.2
Heart ventricle	60	0.03	0.1	Nodose nucleus	27	0.05	0.1	Trigeminal ganglia	16	0.01	0.2
Cervix	60	0.03	0.1	Coronary artery	27	0.03	0.1	Esophagus	16	-0.04	0.4
Nodose nucleus	60	0.02	0.1	Trigeminal ganglia	27	0.03	0.1	Tongue main corpus	16	-0.07	0.3

Adipose omental	60	0.02	0.1	Cervix	27	0.03	0.1	Saphenous vein	16	-0.09	0.2
Testes	60	0.01	0.1	Pituitary gland	27	0.02	0.1	Pharyngeal mucosa	16	-0.09	0.4
Subthalamic nucleus	60	0.00	0.1	Corpus callosum	27	0.02	0.1	Pituitary gland	16	-0.09	0.1
Urethra	60	-0.01	0.2	Tongue main corpus	27	0.01	0.2	Coronary artery	16	-0.10	0.1
Skeletal muscle	60	-0.01	0.1	Trachea	27	0.01	0.1	Urethra	16	-0.11	0.2
Kidney cortex	60	-0.03	0.1	Urethra	27	-0.01	0.1	Vulva	16	-0.11	0.4
Colon cecum	60	-0.05	0.1	Myometrium	27	-0.02	0.1	Adipose omental	16	-0.12	0.1
Prostate gland	60	-0.06	0.1	Adipose omental	27	-0.03	0.1	Prostate gland	16	-0.13	0.2
Thyroid gland	60	-0.07	0.1	Esophagus	27	-0.04	0.1	Ovary	16	-0.14	0.2
Tongue main corpus	60	-0.08	0.1	Vagina	27	-0.05	0.1	Myometrium	16	-0.16	0.1
Coronary artery	60	-0.09	0.1	Stomach fundus	27	-0.06	0.1	Tonsil	16	-0.16	0.3
Kidney medulla	60	-0.10	0.1	Mammary gland	27	-0.06	0.1	Oral mucosa	16	-0.18	0.5
Adrenal gland cortex	60	-0.10	0.1	Adipose subcutaneous	27	-0.06	0.1	Adipose	16	-0.19	0.1
Ovary	60	-0.11	0.1	Stomach cardiac	27	-0.07	0.1	Nipple cross.Section	16	-0.19	0.3
Mammary gland	60	-0.12	0.1	Pharyngeal mucosa	27	-0.08	0.1	Colon cecum	16	-0.20	0.1

Lung	60	-0.12	0.1	Tongue superior part	27	-0.08	0.1	Tongue superior part	16	-0.21	0.3
Stomach cardiac	60	-0.14	0.1	Bronchus	27	-0.10	0.1	Endometrium	16	-0.21	0.2
Stomach fundus	60	-0.15	0.1	Skeletal muscle	27	-0.10	0.2	Mammary gland	16	-0.25	0.2
Adipose	60	-0.15	0.1	Kidney cortex	27	-0.13	0.2	Bronchus	16	-0.25	0.1
Bone marrow	60	-0.16	0.2	Nipple cross.Section	27	-0.13	0.1	Adipose subcutaneous	16	-0.27	0.1
Bronchus	60	-0.16	0.1	Kidney medulla	27	-0.14	0.2	Heart atrium	16	-0.27	0.1
Adipose subcutaneous	60	-0.16	0.1	Salivary gland	27	-0.14	0.1	Stomach fundus	16	-0.27	0.1
Oral mucosa	60	-0.17	0.1	Ovary	27	-0.14	0.1	Thyroid gland	16	-0.27	0.2
Esophagus	60	-0.17	0.1	Lung	27	-0.15	0.1	Kidney medulla	16	-0.28	0.1
Trachea	60	-0.18	0.1	Endometrium	27	-0.16	0.1	Trachea	16	-0.28	0.1
Lymph nodes	60	-0.18	0.1	Adipose	27	-0.17	0.1	Kidney cortex	16	-0.29	0.3
Tongue superior part	60	-0.19	0.1	Oral mucosa	27	-0.17	0.1	Heart ventricle	16	-0.31	0.1
Myometrium	60	-0.19	0.1	Adrenal gland cortex	27	-0.19	0.1	Lymph nodes	16	-0.33	0.2
Stomach pyloric	60	-0.19	0.1	Stomach pyloric	27	-0.19	0.1	Lung	16	-0.33	0.1
Endometrium	60	-0.19	0.1	Prostate gland	27	-0.21	0.1	Stomach cardiac	16	-0.34	0.1

Vulva	60	-0.20	0.1	Lymph nodes	27	-0.21	0.1	Skeletal muscle	16	-0.36	0.2
Pharyngeal mucosa	60	-0.21	0.1	Thyroid gland	27	-0.22	0.1	Adrenal gland cortex	16	-0.36	0.1
Spleen	60	-0.21	0.1	Spleen	27	-0.24	0.1	Stomach pyloric	16	-0.37	0.2
Nipple cross.Section	60	-0.21	0.1	Vulva	27	-0.27	0.1	Testes	16	-0.39	0.1
Liver	60	-0.22	0.1	Testes	27	-0.30	0.1	Salivary gland	16	-0.46	0.1
Salivary gland	60	-0.23	0.1	Bone marrow	27	-0.31	0.1	Spleen	16	-0.49	0.3
Saphenous vein	60	-0.24	0.1	Tonsil	27	-0.33	0.1	Liver	16	-0.55	0.3
Tonsil	60	-0.34	0.1	Liver	27	-0.45	0.1	Bone marrow	16	-0.57	0.3

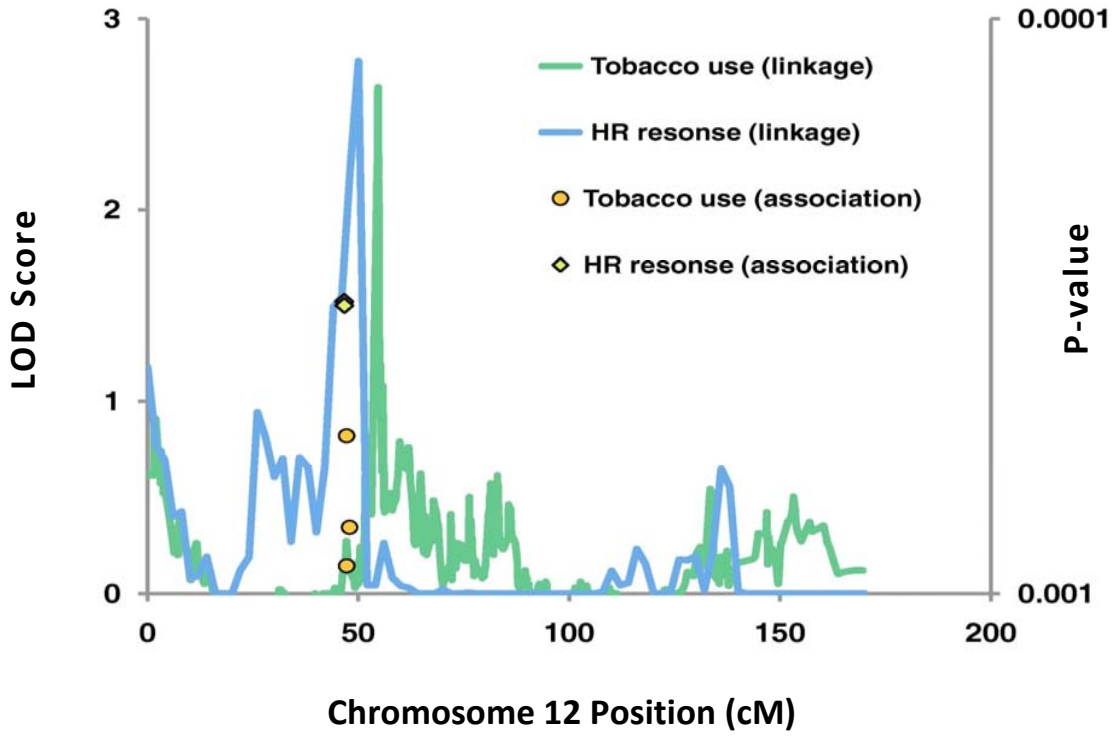

Figure 1. Joint linkage and association analysis identified SNPs inside ITPR2 gene associated to tobacco use and HR response within a common area of linkage for these traits

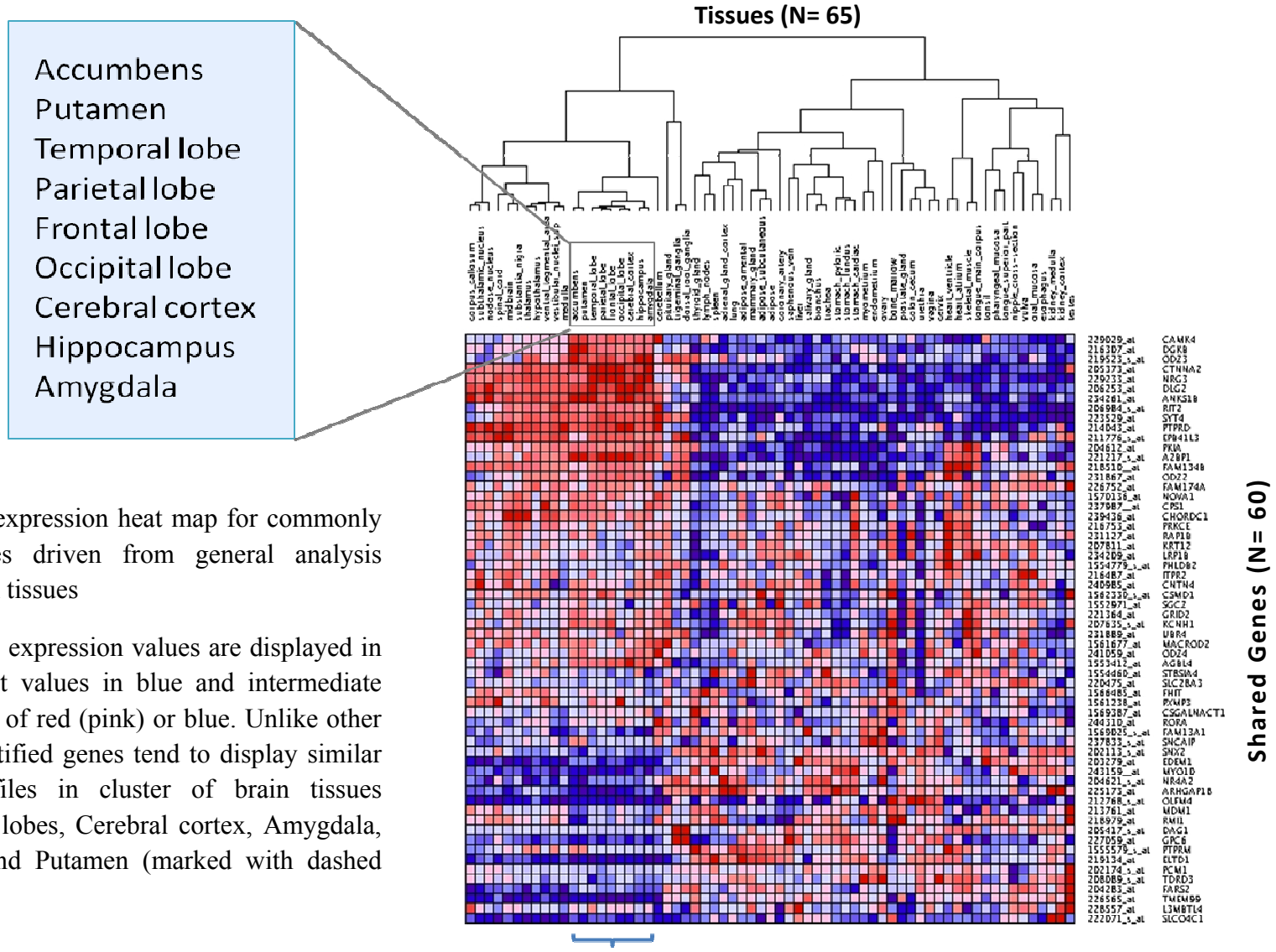

Figure 2. Gene expression heat map for commonly associated genes driven from general analysis across 65 human tissues

The largest gene expression values are displayed in red, the smallest values in blue and intermediate values in shades of red (pink) or blue. Unlike other tissues, the identified genes tend to display similar expression profiles in cluster of brain tissues including Brain lobes, Cerebral cortex, Amygdala, Hippocampus and Putamen (marked with dashed black box).

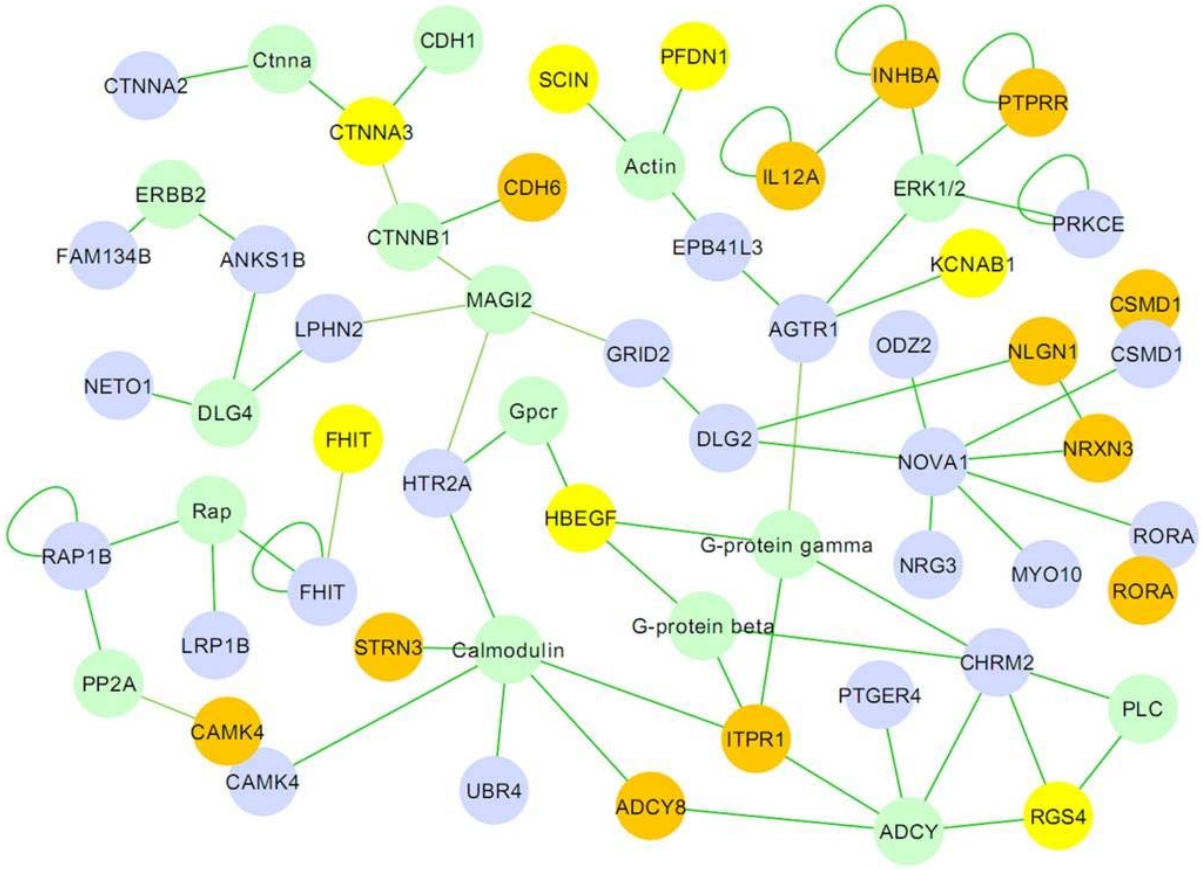

Figure 3. Overview of protein interactions among genes driven from general, sex-specific and hypertension-specific analysis

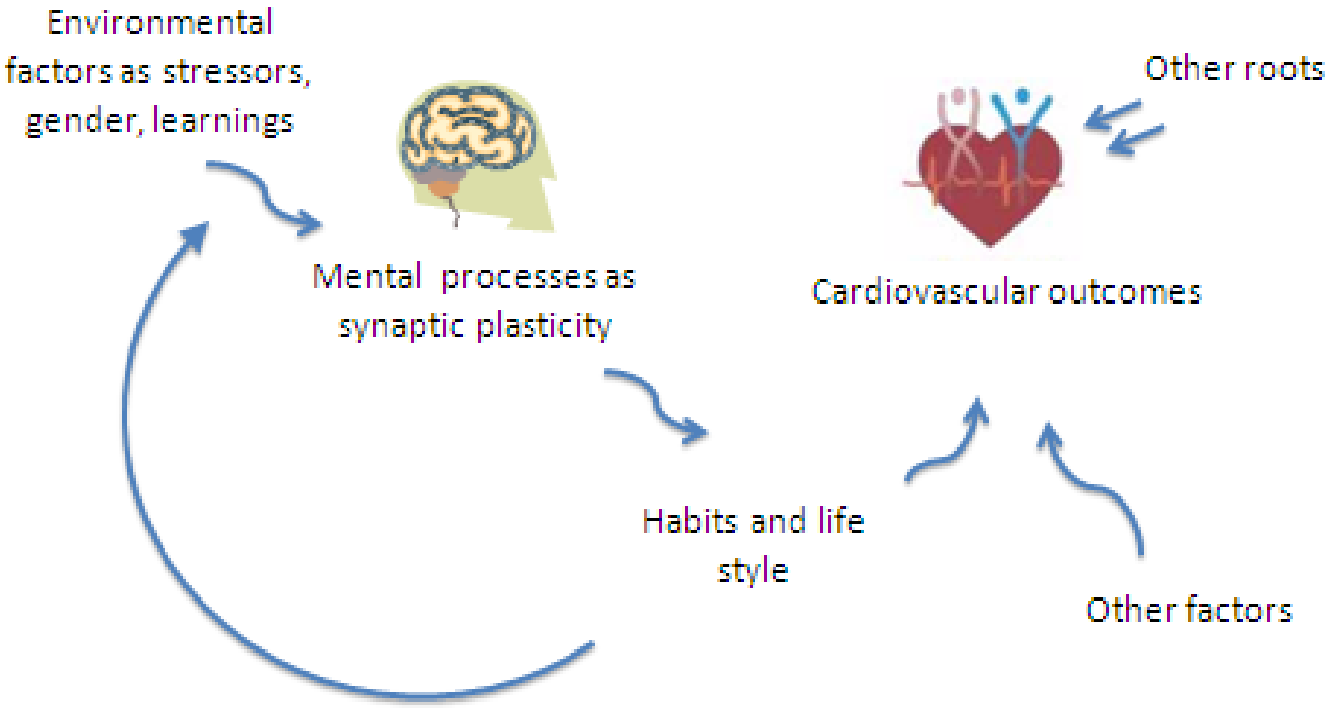

Figure 4. Hypothetical model of interaction among stress, synaptic plasticity, substance use obesity and related-cardiovascular outcomes

Article 2

Probing phenotypic and genotypic relatedness of smoking initiation and persistence with hemodynamic, stress and obesity related traits

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Manuscript is in preparation.

3.1 Abstract

To gain an understanding of interactions between tobacco use with obesity, hypertension and stress, we investigated the phenotypic and genotypic relatedness of smoking initiation and persistence with obesity, hemodynamic and stress related traits in a cohort of 119 multigenerational French Canadian families from founder population of Saguenay–Lac St-Jean region.

Obesity-related anthropometric traits, 24-hour heart rate (HR) and blood pressure (BP) data including response to and recovery from mental stress of mathematical test as well as changes in plasma catecholamine levels after orthostatic test were assessed and combined with smoking data collected during clinical interviews. Following analysis of phenotypic correlations, tobacco use and significantly correlated obesity, hemodynamic and stress related traits with tobacco use ($P < 0.05$) were subjected to univariate and bivariate general, sex- and hypertension-specific family-based genome wide scan using 50K array Affymetrix GeneChip in order to identify the significantly shared loci.

Our findings indicate that the degree and direction of relatedness of tobacco smoking with obesity, hemodynamic and stress related traits vary according to sex and hypertension status; for instance, while in males, current tobacco users were slender compared to never or former tobacco users, there were no such differences in females; moreover, we found several obesity related traits that their correlations with smoking behavior seemingly root in genetic factors rather than smoking effect itself. Shared SNPs reached genome-wide significance ($P < 1.4\times10^{-6}$) were identified between tobacco use with hemodynamic, obesity and stress related traits including SNP, rs679783 inside AGBL4 (Combined $P = 2.3\times10^{-7}$) identified through general analysis; and SNPs, rs947084 inside OBSCN (Combined $P = 1.2\times10^{-6}$) as well as rs36950 inside KCNN2 gene (Combined $P = 8.1\times10^{-8}$) identified through subgroup analysis; in addition, bivariate association analysis further supports the significance of these findings; moreover, rs679783 was significantly associated to both smoking initiation and persistence, rs947084 was significantly associated to smoking initiation and rs36950 was significantly associated to smoking persistence

which point to presence of genetic similarities and differences between current and former tobacco users. In summary, consistent with phenotypic relatedness we identified shared SNPs between tobacco use and significantly correlated obesity, hemodynamic and stress related traits; moreover, our findings underline the importance of subgroup analysis and emphasize the shortcoming of satirical adjustments.

3.2 Introduction

Although tobacco use and stress are related traits but the identity of this relation appears to be complex and paradoxical. Enhancing the positive moods and relief from stress and negative affections are among the main reasons for smoking,[1; 2] it is also reported that concurrent chronic nicotine treatment and stress prevents stress-induced impairment of Long-Term Potentiation pathway (LTP);[3] however, other studies reported that smoking does not reduce, and in some cases enhances the physiological effects of stress.[4; 5]

Tobacco use is also related to hypertension; tobacco smoking temporarily raises blood pressure (BP) and heart rate (HR); nonetheless, the relationship between chronic smoking and development of high blood pressure is unclear and controversial while some studies reported no effect or positive correlation, other studies pointed that smokers have lower BP compare to former and non smokers.[6-8]

Due to numerous health benefits, smoking cession is highly recommended but it is also accompanied with weight gain which indicates an invert relationship between tobacco use and body weight; smokers weight less than nonsmokers of the same age and gender.[9-11] In fact, nicotine administration alters food intake and body weight in both animals and humans and nicotinic receptors have been found in hypothalamic appetite-regulating areas suggesting that centrally mediated actions of nicotine may contribute to the reduced appetite and body weight loss;[12] in contrast, number of studies found that heavy smokers tend to have greater body weight than light smokers or non smokers and smoking is conducive to greater accumulation of visceral fat and increase in waist circumferences.[10; 13; 14]

Phenotypic correlations particularly among complex disorders can be in part due to genotypic relatedness.[15; 16] therefore, the relatedness of obesity, stress and hemodynamic traits with tobacco use may be in part due to genotypic overlaps. In this context, former smoking and current smoking data may help to further investigate whether the observed correlation between tobacco use and obesity, hemodynamic and stress related traits roots in environmental effect (smoking effect) and/or the influence of genetic determinants.

Hence, to investigate the interaction between tobacco use with hypertension, obesity and stress, we probed the phenotypic relatedness of smoking initiation and persistence with hemodynamic, obesity and stress related traits in cohort of families from Saguenay-Lac-Saint-Jean (SLSJ) region.[17] Next, tobacco use and significantly correlated traits ($P < 0.05$) with tobacco use were subjected to univariate and bivariate family-based genome wide scan in order to identify the significantly shared loci.

3.3 Methodology

3.3.1 Family cohort

Families were selected from the population of SLSJ region located in northeastern Quebec[17] which is representing one of the largest founder populations in North America with about 300,000 inhabitants.[18 19]

The founder effect observed in the SLSJ region provides several advantages for gene finding studies for instance, allelic and locus heterogeneity, a key feature of common complex diseases that can obscure the association signal within disease-associated genomic regions is lower in founder population compared to general populations, the likelihood of population stratification that introduces errors and bias the results is also low in a founder population and the large size of LD blocks in these populations reduces the number of markers for whole genome scan studies.[17; 20; 21]

SLSJ population has been already the site of many genetic studies leading to the identification of genes for several monogenomic disorders. A single homozygous mutation was identified in 80% of patients with hereditary tyrosinemia type I,[22] and only three mutations were found in 94% of patients with cystic fibrosis pointing to the reduced genetic heterogeneity in this population.[23] It is also reported that large blocks of ancestral DNA flank the disease mutations in this population;[24; 25] furthermore, the availability of extensive

genealogical records of this population who has computerized genealogical records dating to the original 17th-century settlers provides additional benefits for genetic studies.[17]

Details about families and extensive phenotyping have been described previously.[17; 26-28] Among numerous phenotypes monitored in this cohort, those that are investigated in this study are described.

In summary, families with catholic French Canadian origin were ascertained by the presence of at least one sib pair with hypertension and dyslipidemia. The exclusion criteria included body mass index (BMI) $\geq$ 35 kg/m^2, secondary hypertension, diastolic blood pressure (DBP) >110 mmHg and the use of medication, diabetes mellitus, renal or liver dysfunction, malignancy, pregnancy, and substance abuse. Following the selection of affected sibs, all first- and second-degree relatives aged > 18 years were invited to participate in the study. The recruited population included 119 families (average generations of 2.58) comprising 897 subjects. The study was reviewed and approved by institutional ethics committees of Complexe Hospitalier de la Sagamie, Université du Québec à Chicoutimi, as well as the Centre hospitalier de l'Université de Montréal.

3.3.2 Phenotypes

In the first day of phenotyping, sitting blood pressure was measured and blood samples were obtained to extract DNA from leukocytes for genotyping and measuring other blood factors. Phenotyping for obesity consisted of 3 global and 11 regional measurements of obesity. The global measures included BMI as well as total body fat derived from bioimpedance (RJL Systems Inc) and skinfold measurements. The regional measures included 6 extremity circumferences in upper arm, waist, hip; and proximal, middle and distal thigh; as well as 5 skinfold measurements in biceps, triceps, subscapular, suprailiac and thigh.

Phenotyping for habitual substance use was carried out using questionnaires in about 700 subjects during clinical interviews. Those smokes regularly were grouped as

current tobacco users and non smokers who used to smoke regularly in the past were considered as former tobacco users; in addition, subjects who never or occasionally smoke were grouped as unaffected.

294 subjects without any contraindications were invited for extensive phenotyping and had their antihypertensive drugs withdrawn for 1 week and lipid-lowering agents withdrawn for 1 month. Full phenotyping was performed in a group of 159 normotensive and 135 hypertensive subjects.

In a quiet room with minimal patient interruptions, a postural test was conducted and the plasma epinephrine (EP) and norepinephrine (NE) levels were monitored in blood samples taken from subjects during 60 minutes (min) supine and 10 min standing position, these data were used as biomarkers of response to physical stress.

HR and BP changes to the mental stress of mathematical tests were used as markers of response to and recovery from mental stress. Subjects were asked to sit for 10 min before the test; next, they pass a 2 min arithmetic test. In parallel, HR, DBP and systolic blood pressure (SBP) were measured every 5 min before the test (3 times), every 2 min during the test (2 times) and every 2 min after the test (6 times). Stress response was defined as the differences in the first HR and BP values of math test and the average values of baseline, before the test. Stress recovery was defined as the differences in the first HR and BP values of math test and the average values of resting position, after the test; moreover, the differences between recovery from and response to mental stress was considered as delta stress reactivity.

The mental arithmetic test[29] which was a sequence of simple arithmetic problems were presented to the subjects by a slide projector. Each problem had two components, a simple addition or subtraction followed by a simple multiplication or division. The difficulty level increased and was adjusted to assure some failure for all subjects.

In addition, beginning on the second day of phenotyping, BP and HR were measured every 20 min during the day and every 45 min during the night with an

Accutracker II monitor (Sun Tech Medical Instruments, Inc.) for 24 hours.[17] Using these data, the pulse pressure (PP) was defined as the difference between SBP and DBP and mean arterial pressure (MAP) was defined as one third SBP plus two third DBP.

The inverse normal transformation subsequently was used on quantitative phenotype data to ensure a normal distribution prior to the analysis.

3.3.3 Phenotypic correlations and statistical adjustments

The GEE approach implemented in the GNU R statistical package version 2.6.1 was utilized to perform correlation tests which accounts for familial correlation via a sandwich estimator of the variance under exchangeable correlation.[30 31]

GEEs use the generalized linear model to estimate more efficient and unbiased regression parameters relative to ordinary least squares regression in part because they permit specification of a working correlation matrix that accounts for the form of within-subject correlation of responses on dependent variables of many different distributions.[30 31]

Correlation tests were performed in whole cohort and next separately in males and females as well as in hypertensive and normotensive subjects. In entire sample and hypertensive and normotensive groups, sex, age, alcohol and coffee use were set as covariates in correlation tests and in male and female groups, age, alcohol and coffee use were included as covariates.

3.3.4 Genotypes

Genotyping information has been previously described.[17; 27; 28] In summary, 469 subjects were genotyped with GeneChip® Human Mapping 50K Array Xba240 (Affymetrix) were analyzed in present study. Possible genotyping errors were detected and filtered (~ 0.2 %) using MERLIN package version 1.1.0.[32] An exact test of Hardy-

Weinberg equilibrium (HWE)[33] was performed to remove genotypes that significantly deviate from HWE using PEDSTATS program implemented in MERLIN package. Linkage disequilibrium (LD) among SNPs were calculated using PLINK software version 1.06.[34] SNPs with $r^2 \leq 0.8$, HWE P-value > 0.001 and MAF > 0.05 were used through the analysis. The WGAviewr software version 1.52Z was used to determine the nearby genes around each SNP by specifying up- and down-stream span of 500 kbp.[35]

3.3.5 Genetic analysis

The degree of correlation between tobacco use initiation and persistence with cardiovascular, obesity and stress related traits were calculated. Tobacco use and significantly correlated traits ($P < 0.05$) were subjected to univariate genome wide family-based association tests. SNPs associated to smoking initiation and persistence with P-value $< 10^{-3}$ were preselected. Next we investigated whether these SNPs are associated to at least one significantly correlated trait with tobacco use and achieve the overall association P-value $< 1.4\times10^{-6}$, after Bonferroni correction by the number of SNPs analyzed (34741); furthermore, the detected SNPs were subjected to bivariate family based association tests to investigate association between a SNP and two phenotypes concomitantly.

Since the large portions of variations of these traits are attributed to sex and hypertension status therefore in addition to general, sex- and hypertension-specific family based association tests were also performed. In general and hypertension specific association analysis, age and sex were included as covariates and in sex specific association analysis, only age was used as covariate.

Univariate family-based association tests were performed using the FBAT software version v2.0.2c.[36] Family-based association analysis compared to case-control design is appealing since it tests for association in presence of linkage; hence, findings always imply both linkage and association; beside, family-based association analysis avoids confounding due to model misspecification as well as admixture or population stratification.[37]

To quantify the overall evidence of association of a SNP, Fisher's method was used to combine the individual P-values obtained for each SNP associated to different traits. The method, also known as Fisher's combined probability test, is a technique for meta-analysis or data fusion. Using this method, it is possible to combines P-values from several studies into one test statistic that has a chi-square distribution. The P-value for the X^2 statistic can be inferred from a chi-square table using 2k "degree of freedom", where k corresponds to the number of tests being combined.[38]

Bivariate association analysis was performed using FBAT-GEE implemented in FBAT software version v2.0.2c. FBAT-GEE which is based on GEE approach generalizes univariate family-based association analysis to multivariate scenarios. It can produce a X^2FBAT-GEE statistic, which follows a chi-square distribution. The test can be applied to multiple phenotypes that have different distributions and its degree of freedom corresponds to the number of phenotypes that are tested .[39]

3.4 Results

3.4.1 General characteristics of phenotypes and phenotypic correlations

Former smoking and current smoking were more frequent in males than in females ($P < 10^{-4}$); meanwhile, the prevalence of tobacco use decreased with aging (Table 1 and 2). Descriptive statistics of cardiovascular, obesity and stress related traits distributed by sex, hypertension and tobacco use status along with their correlations with sex, age and hypertension are presented in Table 3 and S1.

None of the studied cardiovascular, obesity and stress related traits were significantly lower in hypertensives than in normotensives (Table S1). Analysis of global and regional obesity-related phenotypes indicated that hypertensive subjects are more obese than normotensives (Table S1) and tobacco users are slender compared to former and never tobacco users (Table 4); moreover, sex specific correlation tests showed that the invert

correlation between tobacco use and obesity measures only exists in males and not in females (Table 5).

Among the overall measurements of obesity, BMI and body fat percentage determined by skinfold were lower in current tobacco users compared to non users (Table 4). Males had higher BMI but lower body fat compared to females ($P < 0.001$) indicating that these traits are sex dependent (Table S1); Following this with sex-specific analysis, we found that , all three global measures of obesity, BMI (Mean±SE; 25.8±0.5 vs 28.2±0.5), body fat percentage derived from skinfold (Mean±SE; 23.8±0.7 vs 27.7±0.5) and body fat percentage determined by bioimpedance (Mean±SE; 21.1±0.9 vs 23.3±0.7) were significantly lower in male smokers than in male non smokers; meanwhile, these traits were not significantly different between current, former and never tobacco users in females (Table 3 and 5). BMI was also lower in tobacco users compared to non tobacco users in both hypertensive and normotensive groups. Body fat percentage determined by bioimpedance was significantly higher in hypertensive smokers compared to hypertensive former smokers ($Z= 3.1$, $P = 0.001$); however, it was lower in normotensive smokers ($Z = -1.7$, $P = 0.04$) than in normotensive former smokers (Table 6).

Tobacco users had lower skinfold in their ticpes, superailiac and thigh as compared to non users (Table 4); assessing the correlation between skinfold measures and sex status indicated that these traits significantly display sexual dimorphism in which females had higher values compared to males (Table S1). Female smokers had higher subscapular skinfold than female non smokers (Mean±SE; 27.9±1.7 vs 24.9±1, $P = 0.01$) while on the other side, male smokers had lower subscapular skinfold compared to (Mean±SE; 21.4±1.3 vs 28.2±1.1, $P < 0.001$) male non smokers. Other skinfold measures including tricpes biccpes, superailiac and thigh skinfold were also significantly lower in male smokers compared to male non smokers (Table 3 and 5). In normotensives, superailiac skinfold ($P = 0.004$) and thigh skinfold ($P = 0.02$) were significantly lower in current tobacco users compared to non users and similar to hypertensive group, the rest of skinfold measures were insignificant among different statuses of tobacco use in this group (Table 6).

Extremity circumferences were significantly different between males and females and between hypertensives and normotensives (Table S1). In entire sample and among males, extremity circumferences including waist, hip, proximal thigh, middle thigh, and distal thigh were significantly lower in current tobacco users compared to never and former tobacco users; however, these measures were not significant among different statuses of tobacco use in females (Table 4 and 5). In both hypertensive and normotensive groups, tobacco users had lower hip and thigh circumferences and higher hip-to-thigh proximal ratio compared to non users (Table 6).

The higher hip-to-thigh proximal ratio appeared to be in part due to significantly reduced thigh circumferences in tobacco users. In fact, the most significantly correlated trait with tobacco use in this study was thigh circumference. All three measures of thigh circumferences were prominently lower in tobacco users compared to never tobacco users with P-values ranging from 10^{-4} to 5×10^{-7}. Former tobacco users also had significantly reduced thigh circumferences than never tobacco users; however, compared to current tobacco users they had higher ($10^{-2} \leq P \leq 10^{-4}$) thigh circumferences (Table 4). Similar patterns were also observed in males, hypertensives and normotensives groups; meanwhile, there were not significant differences among tobacco use statuses in females (Table 5 and 6).

Waist-hip ratio, a commonly used index of upper-body obesity, was not significantly different among tobacco use statuses in females, hypertensives, normotensives as well as in entire sample; however, in males, smokers had significantly lower waist-hip ratio compared to former ($P = 0.02$) and never tobacco users ($P = 0.02$); in addition, there was not major difference between former and never smokers ($P = 0.3$) suggesting use of tobacco is a contributing factor to lower waits-hip ratio in males (Table 4, 5 and 6).

While NE levels were not significantly different among current, former and never tobacco users either in entire sample or specific sub groups (Table 4, 5 and 6). In all, female and normotensive subjects, use of tobacco appeared to increase the EP levels (Table 4, 5 and 6).

Tobacco users had significantly attenuated HR response to mental stress (P = 0.04) and brief difference (P = 0.02) between their SBP response and recovery from mental stress (Delta SBP) as compared to subjects with no history of tobacco use; in addition, tobacco users had shorter HR and SBP recovery from mental stress than former tobacco users (both P = 0.03; Table 4). Delta SBP was also significantly lower in current smokers compared to never tobacco users in females and in normotensives; meanwhile, in normotensives, former smokers had higher SBP response to mental stress compared to non users (Table 3, 5 and 6). DBP recovery from mental stress and response to mental stress were significantly higher in smokers compared to former smokers in hypertensives and also in males (Table 5 and 6); besides, SBP recovery from mental stress (18.9±2.3 vs 24.6±1.8, P = 0.046) as well as response to mental stress (11.5±2.3 vs 15±1.7, P = 0.03) were significantly lower in female smokers compared to former female smokers (Table 5).

Average sitting blood pressures were significantly lower in tobacco users compared to former and non tobacco users in entire sample and among females while in male group only average sitting SBP was significantly lower (121.3±2.8 vs 126.6±2.6, P = 0.04) in current tobacco users than non users (Table 4 and 5). Ambulatory DBP were significantly lower in tobacco users compared to former and non tobacco users in all and among males; meanwhile, male smokers had lower ambulatory HR compared to male non smokers and male former smokers had significantly higher (Mean±SE; 131.7±2.8 vs. 123.5±1.4, P = 0.03) ambulatory SBP compared to male non smokers (Table 4 and 5). In normotensives, use of tobacco appeared to be negatively correlated with mean ambulatory blood pressure (DBP; Z = -2.1 P = 0.02 and SBP; Z = -1.8 P = 0.04); however, hypertensive tobacco users had higher blood pressure (DBP; Z = 1.6 P = 0.06 and SBP; Z = 4 P < 0.001) compared to non users (Table 3 and 6).

3.4.2 Genetic results

Consistent with the observed phenotypic correlations, we identified shared SNPs between tobacco use and significantly correlated obesity, stress and hemodynamic related traits (Table 7).

SNP, rs679783 (HWE P = 0.2, MAF = 0.3) inside AGBL4 gene was significantly associated to current ($P = 6.0\times10^{-4}$) and former tobacco ($P = 1.6\times10^{-4}$) use as compared to subjects with no history of tobacco use; however, the genotypic difference was insignificant between current and former tobacco users (P = 0.7) which indicates genetic similarity between current and former tobacco users and points that this SNP contributes to smoking behavior. Univariate association analysis showed that SNP, rs679783 is also significantly associated to both global and regional measurements of obesity including body mass index, thigh circumferences in proximal, middle and distal as well as waist and hip circumferences ($3.3\times10^{-3} \leq P \leq 7.4\times10^{-5}$). The overall evidence of association for this SNP calculated using Fisher's combined probability test (Combined $P = 2.3\times10^{-7}$) surpassed the Bonferroni threshold ($P = 1.4\times10^{-6}$) for genome wide significance (Table 7). To further investigate the relevance of association of this SNP to smoking and obesity-related traits, we followed the univariate association analysis with bivariate analysis. The resulted bivariate association signals between tobacco use and each of the obesity related traits were all significant for this SNP (P < 0.01;Table 8) which further supports the likely pleiotropic effect of this gene in tobacco use and obesity.

SNP, rs947084 (HWE P = 0.5, MAF= 0.4) inside OBSCN gene was associated to former smokers as compared to current ($P = 5.1\times10^{-4}$) and never tobacco users ($P = 5.8\times10^{-5}$); moreover, the difference between current and never tobacco use (P = 0.5) was insignificant which suggests this SNP is important in smoking initiation but not in smoking persistence and indicates genetic difference between current and former tobacco users; besides, this SNP was trivially associated to each different statuses of tobacco use in females ($P \geq 0.1$) which furthermore points to male specific effect of this marker. SNP, rs947084 was also significantly associated to hip to thigh proximal ratio ($P = 3.2\times10^{-3}$) in

males but not in females (P = 0.3) and combined effects of association of this SNP to tobacco use and hip to thigh proximal ratio was P = 1.2×10^{-6} (Table 7); besides, bivariate association analysis further supports univariate analysis findings (Table 8).

The most significant association signals were obtained for marker, rs36950 located inside KCNN2 gene. This SNP was significantly associated to current tobacco use compared to former (P = 2.7×10^{-5}) and never tobacco use (P = 2.7×10^{-5}) in hypertensives; moreover, the difference between former and never tobacco users was insignificant (P = 0.7) which indicates this SNP contribute to smoking persistence and not smoking initiation; besides, this marker was insignificantly associated to each different statuses of tobacco use in normotensive subjects (P ≥ 0. 8) that points to hypertensive effect of this SNP. We also found that this SNP is significantly associated to SBP recovery from mental stress (P = 1.3×10^{-3} in hypertensives and P = 0.5 in normotensives) as well as mean ambulatory SBP (P = 8.8×10^{-3} in hypertensives and P = 0.9 in normotensives) in hypertensives but not in normotensives (Table 7). The overall evidence of association for this SNP (Combined P = 8.1×10^{-8}) exceeds our genome-wide significance level (P = 1.4×10^{-6}); moreover, bivariate association signals as presented in Table 8 were highly significant for this SNP ($2.5\times10^{-5} \leq P \leq 2.4\times10^{-6}$) which further point the possible pleiotropic effect of this SNP in tobacco use, mental stress and blood pressure. Interestingly, checking the protein interactions showed that both KCNN2 and OBSCN are sharing interactions with CALM1 protein.

3.5 Discussion

In this study, we investigated the phenotypic and genotypic relatedness of tobacco use initiation and persistence with hemodynamic, obesity and stress related traits in cohort of French Canadian families from founder population of SLSJ that provides several advantages over general population, including reduced heterogeneity; longer linkage disequilibrium intervals, as estimated by genetic clock; and importantly, access to their genealogical records.[17; 20; 21]

The short and long-term hemodynamic effect of tobacco smoking has been the subject of many studies; although BP and HR rise immediately after smoking, results from epidemiological studies have generally shown that smokers have lower BP compare to nonsmokers; meanwhile, former smokers have BP similar to those of nonsmokers.[6-8]

Wake HR was significantly higher in tobacco users compared to former and never tobacco users in entire sample and among females and hypertensives which is consistent with previous findings;[8] moreover, in agreement with studies that reported smoking have damping effect on BP; average sitting BP as well as ambulatory DBP were significantly lower in tobacco users compared to former and non tobacco users in entire sample; while, the differences between former and never tobacco users were insignificant pointing to the lowering effect of smoking on BP, similar trends were also observed in males, females and normotensives; however, in hypertensive group, tobacco users had higher DBP as well as SBP compared to non users which suggest there might be hypertension specific factors (e.g. genetic factors) that modify effect of tobacco smoking on blood pressure.[6; 7] For instance, as it is also observed in our cohort, smoking increases the epinephrine level in the body; [40; 41] however, it has been documented that compared to normotensives, the elevated level of epinephrine significantly enhances the cardiac response in hypertensives.[42]

Overall, analysis of both global and regional measurements of obesity indicated that current tobacco users are slender compared to never or former tobacco users; these findings are consistent with numerous studies that indicated smokers tend to have lower body weight than do non-smokers.[10; 11; 43; 44]Apart from thigh circumferences and thigh skinfold, other obesity measures were insignificant between former and never tobacco users which furthermore support the notion that smoking cession is associated with body weight gain.[45; 46] Thigh circumferences were significantly lower in current tobacco users compared to former and never tobacco users; moreover, former tobacco users had lower thigh circumferences than non users. These findings suggest the observed differences in thigh circumference may have tobacco related roots as well as genetic underpinnings that are shared among current and former tobacco user.

Similar trends were observed in males and normotensives; however, in females obesity related measures were not significantly different among tobacco use statues and even female smokers had higher subscapular skinfold than female non smokers, the fact that smoking cession or initiation is not associated to weight gain in females is interesting since studies have shown that compared to men, women are more concern about unwanted weight gain as a result of smoking cession and among female smokers, many smoke to control their weight; in addition, they are more likely to resume smoking to lose weight. [47; 48] Nevertheless, our result supports previous findings in which weight gain did not necessarily follow smoking cessation in females. [13; 49]

It has been suggested that waist circumference or waist-to-hip ratio, predictors of intra-abdominal visceral fat is higher in smokers than in nonsmokers;[10; 13; 14] however, we found that tobacco users have lower waist and hip circumferences compared to former and never tobacco users, these differences were not significant between former and never tobacco users suggesting use of tobacco decreases waist and hip circumferences, besides; the waist-hip ratio, a commonly used index of upper-body obesity, was not significantly different among tobacco use statuses, similar trends were also observed in normotensives and hypertensives. In male group, smokers had even significantly lower waist-hip ratio compared to former and never tobacco users; in addition, there was no significant difference between former and never smokers suggesting use of tobacco is contributing to lower waits-hip ratio in males. Relative to hypertension, these findings are interesting, since central obesity is positively correlated with hypertension [50; 51]; therefore, the observed lower blood pressure among tobacco users in our cohort can be also due to lower intra-abdominal visceral fat in tobacco users compared to non tobacco users.

The effect of smoking on hemodynamic reactions to psychological stress exposures is not clear. While some studies have found smoking has additive effect on hemodynamic reactivity to mental stress, [52-54] others reported attenuated response to mental stress in smokers compared to non smokers or no significant differences;[8; 55-57] meanwhile, studies have found sex-smoking interaction on response to mental stress.[58; 59]

Overall, we found that, tobacco users have significantly attenuated HR response to mental stress and brief difference between their SBP response and recovery from mental stress as compared to subjects with no history of tobacco use; in addition, tobacco users had shorter HR and SBP recovery from mental stress than former tobacco users.

Our findings are in agreement with the growing body of literature showing that smokers have blunted reactivity to mental stress.[8; 55; 56] These results also suggest in addition to response to mental stress which is usually used by most of the studies that compare hemodynamic reactivity in smokers and non-smokers, assessment of recovery from mental stress and the difference between response and recovery can provide additional insights into influence of smoking on hemodynamic reactions to psychological stress.

Subgroup analysis indicated that there are hypertension and sex differences in hemodynamic reactivity to psychological and physiological stressors; for instance, in hypertensive and in male groups, DBP recovery from as well as response to mental stress were significantly higher in smokers compared to former smokers but not in females and normotensives, our findings are consistent with those that reported male smokers are more reactive than male non-smokers in their diastolic blood pressure response to mental arithmetic test; [58; 60] in addition, we found that in male group, SBP recovery from and response to mental stress were significantly higher in former smokers compared to never smokers while this trend was opposite in females; moreover, in agreement with previous findings,[61] these measurements were significantly lower in female smokers compared to former smokers.

While NE levels were not significantly different among tobacco use statuses either in entire sample or specific sub groups. In all, females and normotensives use of tobacco appeared to increase the EP levels. This is consistent with those studies that reported tobacco use increases the level of EP.[40; 62] In fact, nicotine binds to ganglion type nicotinic receptors in the adrenal medulla and increases flow of epinephrine and this appears to be in part responsible for stimulating effects of tobacco use.[40; 41; 62; 63]

In present study, no account was taken of the extent of smoking. Although smoking is sometimes underestimated in self-reports, particularly among former smokers,[64] several studies found that smoking information collected using questionnaires can be reasonably reliable and agree to biochemical assessments.[65; 66] Nonetheless, it would have strengthened our study if additional detailed information regarding duration and intensity of smoking behavior as well as biochemical measures to verify smoking behavior were available.

Phenotypic correlation particularly among complex disorders appears to be in part due to genotypic relatedness;[15; 16; 67; 68] therefore, the observed correlation between tobacco use with obesity, hemodynamic and stress related traits may be partly due to genetic overlaps.[15; 16] Identification of such pleiotropic genes can provide several benefits for health programs,[16; 69-71] such as providing additional insights into mechanisms of comorbidity of these phenotypes, cross-utilization of drugs and therefore expanding application of current medications, reducing the possible drug side effects and eventually development of more rational therapeutic approaches.

Using both univariatre and bivariat family-based association analysis, we searched for common loci between tobacco use and significantly correlated obesity, hemodynamic and stress related traits with tobacco use. We found three commonly associated SNPs to tobacco use and significantly correlated traits with tobacco use inside KCNN2, OBSCN and AGBL4 genes, findings which were further supported by bivairaie association analysis. KCNN2 gene was shared among tobacco use, mean ambulatory SBP as well as SBP response to and recovery from mental stress; while, AGBL4 and OBSCN genes were shared between obesity-related traits and tobacco use.

The most significant association signal was obtained for KCNN2 gene which is a member of the calcium-activated potassium channel family and appears to regulate neuronal excitability by contributing to the slow component of synaptic after hyper polarization. Interestingly, this gene has been already implicated in stress related traits; for instance, over-expression of this gene in the basolateral amygdala reduces anxiety and stress-induced corticosterone secretion at a systemic level.[72] It has also been reported that

KCNN2 negatively regulates the excitation of enteric neurons via functional interactions with nicotinic acetylcholine receptors;[73] moreover, this gene is involved in cardiac repolarization in atrial myocytes[74; 75] which further support pleiotropic characteristic identified in present study for this gene. OBSCN encoded protein belongs to the family of giant sacromeric signaling proteins and have role in the organization of myofibrils during assembly, it mediates interactions between the sarcoplasmic reticulum and myofibrils and It has been implicated in hypertrophic cardiomyopathy.[76] Interestingly both KCNN2 and OBSCN have been earlier shown to interact with CALM1 protein suggesting these proteins may have function in similar physiological processes.[77-79] AGBL4 has been already implicated in substance use;[80; 81] however, little is known about AGBL4 function and it may play a role in the processing of tubulin.

SNP inside KCNN2 gene was significantly associated to tobacco use, stress and blood pressure in hypertensives and not in normotensives; additionally, SNP inside OBSCN gene was commonly associated to tobacco use and hip thigh proximal ratio in males and not in females. Detection of such specific loci may be due to their sex- and hypertension-specific effects or co-segregation with epistatic sex- and hypertension specific genetic factors that modify their effects;[28; 51; 82; 83] consistently, we found that large portions of variations of studied traits are attributed to sex and hypertension status; moreover, we have already reported sex- and hypertension-differences in the heritabilities of many of these traits.[28; 82] Therefore, by increasing genetic homogeneity, subgroup analysis can uncover genetic factors that otherwise would remain obscured.

Inclusion of former smokers in this study allows us to determine whether smoking cession is associated with a similar magnitude of change to that typical of non smokers and also if the shared genetic factors between former and current tobacco users contribute to the differences. In this regard we found several obesity related traits that their relatedness with smoking behavior seemingly are mediated by genetic factors. Thigh skinfold in whole cohort subscapular skinfold in females, superaialic skinfold and body fat percentage in normotensives along with hip thigh proximal ratio in both normotensives and hypertensives

were significantly correlated with current and former tobacco users as compared to subjects with no history of tobacco use; moreover, the differences between current and former tobacco users were not significant for these traits suggesting that the observed correlations probably roots in genetic factors that are shared between former and current tobacco users rather than smoking effect itself.

Moreover, our genetic findings highlight the presence of genetic similarities and differences between current and former tobacco users. SNP, rs679783 was significantly associated to both former and current tobacco use while rs947084 was significantly associated to smoking initiation and SNP, rs36950 was significantly associated to smoking persistence. Consistent with these findings it is also reported that genetic factors differently contribute to the determination of smoking initiation and persistence;[84] moreover, we have also previously reported that genetic factors account for 61% variability of smoking initiation and 46% variability of smoking persistence.

In summary consistent with phenotypic relatedness we identified shared SNPs between tobacco use and significantly correlated obesity, hemodynamic and stress related traits; moreover, our findings underline the importance of subgroup analysis and emphasize the shortcoming of satirical adjustments.

Although in some gene-environment studies, tobacco use is considered as an environmental factor, we found several obesity related traits that their correlations with smoking behavior appear to root in genetic factors rather than smoking effect itself; in addition, consistent with phenotypic relatedness we identified shared SNPs between tobacco use and significantly correlated obesity, hemodynamic and stress related traits. These findings point that tobacco use as an environmental factor has its own genetic underpinnings and phenotypic correlation may be an indicator of genotypic relatedness; in addition, this study raises implications for gene environment studies that include tobacco use as an environmental factor and points to considering subgroup analysis in genetic studies and personalized medicine programs.

Author Contributions

P.H., J.T., D.G., T.A.K. and A.W.C.J. established family cohort and conducted sample selection, phenotyping, genotyping and project management. P.H. is director of project and supervised the entire study. M.N. did the quality checks of phenotype and genotype data; calculated heritabilities; carried out statistical analysis, genetic scans, pathway/network analysis and, insilico-functional annotation and; drafted the manuscript. O.Š. and J.T. contributed to the conception and design of study.

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Table 1. Characteristics of tobacco use statuses

Characteristic	Never tobacco users	Former tobacco users	Current tobacco users	All
Age (Mean±SE; N)	48.5±0.9; 290	54.4±0.8; 225	46.9±1; 182	50±0.6; 697
Females (N)	190	101	86	377
Males (N)	100	124	96	320
Hypertensives (N)	158	141	75	374
Normotensives (N)	132	84	107	323

Table 2. Distribution of tobacco use[1] traits by sex and hypertension status along with their correlations[2] with sex, age[3] and hypertension[4]

Trait	Females				Males				Sex (females)		Aging		Hypertension (hypertensives)		Total
	Unaffected		Affected		Unaffected		Affected								
	Nor[5]	Hyp[6]	Nor	Hyp	Nor	Hyp	Nor	Hyp	Z	P	Z	P	Z	P	
Current vs. never tobacco users	17.4	22.9	10.6	7.6	10.6	10.6	12.1	8.3	-4.1	**< 1E-04**	-0.9	0.2	-3.0	**0.001**	472
Current vs. former tobacco users	8.6	16.2	12.3	8.8	12.0	18.4	14.0	9.6	0.3	0.4	-4.5	**< 1E-04**	-3.2	**0.0006**	407
Former vs. never tobacco users	15.9	21.0	6.8	12.8	9.7	9.7	9.5	14.6	-4.8	**< 1E-04**	3.4	**0.0004**	-0.6	0.3	515

[1] Data are in percentage.
[2] Correlation test was done using GEE method. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa.
[3] Correlation model is substance use ~ sex + age.
[4] Correlation model is hypertension status ~ sex + age + substance use.
[5] Normotensives
[6] Hypertensives

Table 3. Descriptive statistics of obesity, stress and hemodynamic related traits distributed by sex, hypertension and tobacco use statuses

Trait	Males						Females						All
	Normotensives			Hypertensives			Normotensives			Hypertensives			
	Never tobacco users	Former tobacco users	Current tobacco users	Never tobacco users	Former tobacco users	Current tobacco users	Never tobacco users	Former tobacco users	Current tobacco users	Never tobacco users	Former tobacco users	Current tobacco users	
	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)	Mean±SE (N)
BMI (kg/m2)	28.1±0.8 (49)	26.4±0.6 (49)	24.7±0.6 (54)	28.3±0.6 (48)	28.7±0.5 (73)	27.3±0.8 (39)	24.3±0.5 (80)	25±0.7 (35)	25.4±0.7 (50)	27.2±0.5 (104)	27.5±0.7 (63)	27.1±0.9 (35)	26.7±0.2 (679)
Waist Hip ratio	0.9±0 (48)	1±0 (47)	0.9±0 (56)	1±0 (50)	1±0 (75)	1±0 (37)	0.8±0 (78)	0.8±0 (35)	0.8±0 (48)	0.8±0 (100)	0.9±0 (63)	0.8±0 (36)	0.9±0 (673)
Waist circumference (cm)	95.6±1.4 (48)	95.9±1.4 (47)	88.4±1.7 (56)	99.2±1.6 (50)	100.7±1.4 (75)	97.7±2.2 (37)	79.5±1.5 (79)	79±2.3 (35)	81.7±1.7 (48)	86±1.4 (100)	87.8±1.9 (63)	86.6±2.5 (36)	89.6±0.6 (674)
Hip circumference (cm)	100.8±0.8 (48)	99.4±0.9 (47)	94.8±1 (56)	101.9±1.1 (50)	101±0.9 (75)	100±1.5 (37)	98.8±1.2 (78)	99.5±1.4 (35)	99.5±1.5 (48)	101.6±1.1 (100)	102.9±1.4 (63)	102.5±1.9 (36)	100.3±0.4 (673)
Hip Thigh Proximal ratio	1.7±0 (48)	1.8±0 (46)	1.7±0 (54)	1.7±0 (47)	1.8±0 (71)	1.8±0 (35)	1.7±0 (78)	1.7±0 (35)	1.7±0 (48)	1.7±0 (96)	1.7±0 (58)	1.7±0 (32)	1.7±0 (648)
Thigh proximal circumference (cm)	60.6±0.8 (48)	57±0.7 (46)	54.6±0.8 (55)	60.7±1 (47)	57.2±0.6 (71)	57.4±1.2 (35)	59.2±0.9 (78)	58±1 (35)	59.7±1.2 (48)	58.4±0.6 (96)	58.6±0.8 (58)	57.6±1.1 (32)	58.3±0.3 (649)
Thigh mid circumference (cm)	55.2±0.8 (48)	52.2±0.7 (47)	51.4±0.9 (54)	55±0.8 (47)	52.8±0.6 (71)	52.4±1 (36)	52.1±0.7 (78)	52.1±0.9 (35)	52.9±0.9 (48)	52.6±0.6 (98)	52.8±0.8 (59)	51.1±1.2 (32)	52.7±0.2 (653)
Thigh distal circumference (cm)	41.5±0.5 (48)	40.3±0.7 (46)	38.6±0.5 (55)	41.6±0.5 (47)	40.4±0.5 (71)	40.5±0.8 (35)	39.8±0.5 (78)	39.6±0.7 (35)	40±0.7 (48)	40.5±0.5 (97)	40.3±0.6 (58)	39.8±0.9 (32)	40.2±0.2 (650)

Skinfold bicep1 (mm)	18.2±1.7 (47)	18.5±2 (44)	14.9±1.5 (53)	20.2±1.8 (43)	18.1±1.7 (65)	18.8±2.9 (31)	21±1.4 (76)	21±2.1 (30)	25±2.4 (44)	23.9±1.3 (81)	26.5±2 (47)	22.3±2.7 (23)	20.7±0.5 (584)
Skinfold bicep2 (mm)	18.8±1.7 (47)	18.7±2.1 (44)	15.1±1.6 (53)	20.1±1.8 (43)	18.3±1.7 (65)	19.1±2.9 (31)	21.3±1.5 (76)	21±2.1 (30)	25.4±2.4 (44)	23.9±1.3 (81)	27.4±2.1 (47)	22.5±2.8 (23)	21±0.6 (584)
Skinfold bicep3 (mm)	18.8±1.8 (47)	18.9±2.1 (44)	15.1±1.6 (53)	20.1±1.8 (43)	18.1±1.6 (65)	18.7±2.9 (31)	21.6±1.5 (76)	21.4±2.2 (30)	25.1±2.4 (44)	24.2±1.3 (81)	27.2±2.1 (46)	23.1±2.9 (23)	21±0.6 (583)
Mean skinfold biceps (mm)	18.6±1.7 (47)	18.7±2.1 (44)	15.1±1.5 (53)	20.1±1.8 (43)	18.1±1.7 (65)	18.9±2.9 (31)	21.3±1.5 (76)	21.1±2.1 (30)	25.2±2.4 (44)	24±1.3 (81)	27.1±2 (47)	22.6±2.8 (23)	20.9±0.5 (584)
Skinfold triceps1 (mm)	26.3±1.8 (47)	24.8±2.1 (44)	20.7±1.8 (53)	27.7±1.8 (43)	24.4±1.6 (65)	25.3±3 (31)	29.1±1.2 (76)	30.7±2 (31)	31.6±2.2 (44)	34.1±1.2 (81)	34.5±1.8 (47)	33±2.8 (23)	28.5±0.5 (585)
Skinfold triceps2 (mm)	26.5±1.8 (47)	25.2±2.2 (44)	21±1.8 (53)	27.6±1.8 (43)	24.2±1.5 (64)	25.3±3 (31)	29.4±1.3 (76)	30.9±2 (31)	31.8±2.2 (44)	34.4±1.2 (81)	35.3±1.8 (47)	33.2±2.8 (23)	28.7±0.5 (584)
Skinfold triceps3 (mm)	26.7±1.8 (47)	25.2±2.2 (44)	20.7±1.7 (53)	27.8±1.8 (43)	24.1±1.5 (64)	25.2±3 (31)	29.4±1.3 (76)	31.1±2 (31)	32.1±2.2 (44)	34.3±1.2 (80)	35.6±1.8 (47)	33.5±2.8 (23)	28.8±0.5 (583)
Mean skinfold triceps (mm)	26.5±1.8 (47)	25±2.1 (44)	20.8±1.7 (53)	27.7±1.8 (43)	24.7±1.6 (65)	25.3±3 (31)	29.3±1.3 (76)	30.9±2 (31)	31.8±2.2 (44)	34.4±1.2 (81)	35.1±1.8 (47)	33.2±2.8 (23)	28.7±0.5 (585)
Skinfold subscapular1 (mm)	27.1±1.4 (47)	22.8±1.5 (44)	19.6±1.5 (53)	29.3±1.8 (43)	25.5±1.2 (63)	24.4±2.2 (30)	23.5±1.5 (76)	22±1.7 (31)	27.2±2.3 (44)	25.9±1.2 (80)	30.8±2 (47)	28.4±2.4 (23)	25.4±0.5 (581)
Skinfold subscapular2 (mm)	26.9±1.4 (47)	23±1.5 (44)	19.6±1.5 (53)	29.1±1.8 (43)	25.5±1.1 (63)	24.7±2.3 (30)	23.7±1.5 (76)	22.4±1.7 (31)	27.7±2.3 (44)	25.9±1.2 (80)	31.1±2.1 (47)	28.8±2.5 (23)	25.5±0.5 (581)
Skinfold subscapular3 (mm)	27.3±1.4 (47)	23.1±1.5 (44)	19.7±1.5 (53)	29.5±1.8 (43)	25.6±1.2 (63)	24.5±2.3 (30)	23.9±1.5 (76)	22.4±1.7 (31)	27.6±2.3 (44)	26.1±1.3 (80)	31.2±2.1 (47)	28.8±2.5 (23)	25.6±0.5 (581)
Mean skinfold subscapular (mm)	27.1±1.4 (47)	23±1.5 (44)	19.6±1.5 (53)	29.3±1.8 (43)	25.6±1.2 (63)	24.5±2.3 (30)	23.7±1.5 (76)	22.2±1.7 (31)	27.5±2.3 (44)	26±1.2 (80)	31±2 (47)	28.7±2.5 (23)	25.5±0.5 (581)

Skinfold suprailiac1 (mm)	27.6±1.9 (46)	19.1±1.6 (44)	18.5±1.8 (53)	26.7±2.3 (42)	21.4±1.5 (64)	22±2.2 (30)	23.9±1.6 (76)	23±2 (31)	24.6±2.4 (42)	24.9±1 (80)	28.6±1.7 (47)	27.6±2.2 (23)	23.8±0.5 (578)
Skinfold suprailiac2 (mm)	27.6±1.9 (46)	19.6±1.6 (44)	18.7±1.9 (53)	26.8±2.3 (42)	21.7±1.5 (64)	22.1±2.2 (30)	23.8±1.6 (76)	23.4±1.9 (31)	24.4±2.4 (42)	25.3±1.1 (80)	29±1.7 (47)	27.8±2.2 (23)	24±0.5 (578)
Skinfold suprailiac3 (mm)	27.5±1.9 (46)	19.6±1.6 (44)	19±1.9 (53)	27±2.2 (42)	21.8±1.5 (64)	22.3±2.2 (30)	23.7±1.6 (76)	23.4±2 (31)	25±2.5 (42)	25.5±1.1 (80)	29.1±1.7 (47)	28±2.2 (23)	24.2±0.5 (578)
Mean skinfold suprailiac (mm)	27.6±1.9 (46)	19.4±1.6 (44)	18.7±1.9 (53)	26.8±2.3 (42)	21.6±1.5 (64)	22.1±2.2 (30)	23.8±1.6 (76)	23.3±2 (31)	24.7±2.4 (42)	25.2±1.1 (80)	28.9±1.7 (47)	27.8±2.2 (23)	24±0.5 (578)
Skinfold thigh1 (mm)	31.8±2.1 (45)	28.5±2.6 (43)	23.9±2.1 (51)	29.1±2.5 (42)	23.8±1.6 (62)	26.3±2.9 (30)	38.3±1.5 (69)	39.2±2.1 (29)	40.4±2.3 (37)	43±1.4 (73)	43.5±1.9 (45)	40.8±2.9 (22)	34±0.7 (548)
Skinfold thigh2 (mm)	32±2.1 (45)	28.9±2.7 (43)	24.1±2.1 (51)	29.6±2.5 (42)	24±1.6 (62)	26.5±2.9 (30)	38.6±1.5 (69)	38.9±2.2 (29)	40±2.4 (36)	43.3±1.4 (73)	43.6±1.9 (45)	41.1±3 (22)	34.2±0.7 (547)
Skinfold thigh3 (mm)	32.4±2.2 (45)	27.9±2.6 (42)	24.4±2.1 (51)	29.7±2.6 (42)	24.1±1.7 (62)	26.4±3 (30)	39.1±1.5 (69)	39.1±2.2 (29)	40.1±2.4 (36)	43.8±1.4 (73)	44±1.9 (45)	41.4±3 (22)	34.4±0.7 (546)
Mean skinfold thigh (mm)	32.1±2.1 (45)	28.7±2.7 (43)	24.1±2.1 (51)	29.5±2.5 (42)	24±1.6 (62)	26.4±2.9 (30)	38.7±1.5 (69)	39.1±2.2 (29)	40.6±2.4 (37)	43.4±1.4 (73)	43.7±1.9 (45)	41.1±3 (22)	34.2±0.7 (548)
Body fat percentage bioimpedance	21.8±1.1 (41)	25.1±1.4 (34)	18.5±1.1 (44)	24.9±1 (36)	25.6±1.1 (57)	25.4±1.3 (26)	30.2±1.2 (66)	31±1.8 (25)	30.8±1.4 (36)	36.8±1.2 (69)	37.4±1.8 (41)	36.4±2.7 (16)	28.7±0.5 (491)
Body fat (%)	27.6±0.8 (46)	25.2±0.9 (44)	23.2±0.9 (53)	27.9±0.8 (42)	25.8±0.6 (63)	24.9±1.1 (29)	36.2±0.9 (76)	36.6±1.2 (30)	36.9±1.2 (42)	38.6±0.6 (80)	40.1±1 (47)	38.9±1.2 (23)	32±0.4 (575)
Supine EP (pg/ml)	45.2±3.4 (19)	41.9±6.3 (17)	43.2±2.6 (20)	46.2±6.5 (13)	39.8±3.8 (15)	44.8±8 (10)	44.4±3.3 (35)	37.1±4.3 (10)	43.3±3.6 (16)	37.9±3 (22)	40.8±4.1 (12)	58.8±22.2 (3)	42.7±1.3 (192)
Standing EP (pg/ml)	59.3±9.2 (18)	48.3±7.5 (15)	61.8±7.7 (20)	55.1±7.2 (13)	50.1±5.5 (15)	50.6±7.2 (8)	47.9±4.1 (34)	42.5±8.4 (9)	62.6±7.8 (19)	36.6±3.1 (22)	38.1±3.8 (9)	51.3±11.7 (3)	50.8±2 (185)

Response EP (pg/ml)	14.5±9.6 (18)	6.1±6.9 (15)	18.6±7.6 (20)	6.3±8.8 (12)	10.2±4.7 (12)	3.3±6.8 (8)	4.8±4.4 (33)	3.6±5.7 (9)	23.1±9.7 (16)	0.3±2.9 (18)	0.1±7.2 (9)	-7.5±10.5 (3)	8.6±2.2 (173)
Supine NE (pg/ml)	177.5±19 (19)	152.8±13.5 (17)	176.8±12 (20)	170.5±22 (16)	167.4±17.3 (18)	162.9±24.4 (10)	170.2±11.1 (36)	185.2±15.5 (11)	164.6±12.5 (20)	164.8±11.2 (26)	161.4±22.6 (12)	226.6±74.1 (3)	169.4±4.7 (208)
Standing NE (pg/ml)	355.3±27.4 (18)	381.7±47.8 (16)	442.8±44.3 (20)	418.4±45 (16)	398.7±41.4 (19)	438.9±57.4 (10)	395.1±23.1 (36)	412.9±39.1 (11)	372.5±26.1 (20)	412.3±33.5 (26)	382.2±58.6 (12)	504±74.3 (3)	401.2±11.2 (207)
Response NE (pg/ml)	180.4±22.3 (18)	235.4±40 (16)	266±40.9 (20)	247.9±32.5 (16)	231.9±33.1 (18)	276.1±35 (10)	224.9±17.3 (36)	227.7±28.7 (11)	207.8±21.9 (20)	247.5±27.5 (26)	220.8±40.7 (12)	277.4±8.9 (3)	232.6±8.9 (206)
DBP recovery to mental stress (mmHg)	10.6±1.4 (22)	9.7±1.8 (18)	11.1±1.5 (23)	9.9±1.7 (19)	7.3±1.1 (23)	11.4±2.3 (13)	11±1.2 (37)	9.7±1.7 (15)	10.6±1.4 (19)	13.1±1.4 (27)	14.7±1.3 (19)	11.9±4.1 (4)	10.9±0.4 (239)
Delta DBP (mmHg)	0.6±0.5 (22)	0.6±1.1 (18)	1.4±0.7 (23)	0.8±1.1 (19)	0.8±0.7 (23)	-0.7±1.2 (13)	0.9±0.7 (37)	-0.1±1.2 (15)	0±0.6 (19)	1.1±1 (27)	1.9±1.1 (19)	2.8±0.6 (4)	0.8±0.3 (239)
DBP response to mental stress (mmHg)	10±1.5 (22)	9.1±2.2 (18)	9.7±1.5 (23)	9.2±1.7 (19)	6.5±1.3 (23)	12.2±2.3 (13)	10.1±1.3 (37)	9.8±1.8 (15)	10.6±1.2 (19)	12±1.3 (27)	12.8±1.5 (19)	9.1±4.6 (4)	10.1±0.5 (239)
SBP recovery from mental stress (mmHg)	21.1±2.3 (22)	24.8±2.9 (18)	20.6±2.5 (23)	23±2.4 (19)	30.6±2.8 (23)	26.1±2.6 (13)	19.9±2 (37)	18.9±2.9 (15)	17.9±2.5 (19)	31±2.3 (27)	29±1.6 (19)	24.9±4.5 (3)	23.9±0.8 (238)
Delta SBP (mmHg)	7.2±1.1 (22)	8.4±1.6 (18)	6.1±1 (23)	10±2.3 (19)	9.1±1.2 (23)	7.9±1.9 (13)	8.3±0.9 (37)	5.4±1.2 (15)	6.6±0.9 (19)	11±1 (27)	12.9±2.5 (19)	12.2±3.2 (3)	8.6±0.4 (238)
SBP response to mental stress (mmHg)	13.9±2 (22)	16.4±3 (18)	14.4±2.4 (23)	13±2.5 (19)	21.5±2.4 (23)	18.3±3.2 (13)	11.6±1.9 (37)	13.5±3.1 (15)	11.3±2.6 (19)	20±2.5 (27)	16.1±2 (19)	12.8±6.7 (3)	15.3±0.8 (238)
HR recovery to mental stress (beast/min)	6.7±2.2 (22)	7.3±1.6 (18)	5.9±2.2 (23)	6.2±1.4 (19)	9.1±2.1 (23)	8.1±2.7 (13)	8.3±1.7 (37)	13±5.3 (16)	5.9±0.9 (19)	11.9±1.7 (27)	9.6±1.7 (19)	10±4.8 (4)	8.4±0.7 (240)
Delta HR (beast/min)	-0.8±0.5 (22)	0±0.8 (18)	-0.1±0.7 (23)	-1.3±1 (19)	0.6±0.8 (23)	0.2±1.4 (13)	1.6±0.8 (37)	0.7±0.8 (16)	0.1±0.9 (19)	1.6±0.8 (27)	-0.2±0.9 (19)	0.4±1.1 (4)	0.3±0.3 (240)

HR response to mental stress (beast/min)	7.5±2.1 (22)	7.3±1.6 (18)	6±2 (23)	7.5±1.4 (19)	8.5±1.8 (23)	7.9±2.4 (13)	6.7±1.5 (37)	12.3±5.4 (16)	5.9±1.1 (19)	10.3±1.6 (27)	9.8±1.6 (19)	9.6±4.4 (4)	8.1±0.6 (240)
Average sitting DBP (mmHg)	74.2±1.8 (21)	79.3±2.1 (19)	72.4±1.7 (23)	83.9±2.2 (19)	88.4±2.3 (23)	82.7±3.4 (13)	70±1.5 (35)	70.3±1.6 (16)	66.8±2 (20)	77.3±1.8 (27)	80.1±2.7 (18)	78.2±2.4 (5)	76.4±0.7 (239)
Average sitting SBP (mmHg)	119.6±2.1 (21)	122.2±2.7 (19)	113.6±1.8 (23)	134.3±4.3 (19)	139.2±3.6 (23)	134.9±5.4 (13)	110.8±1.9 (35)	108.5±2 (16)	105.6±2.7 (20)	133.7±3.3 (27)	135.4±4.9 (18)	139.5±7.1 (5)	123.1±1.2 (239)
Average sitting HR (beast/min)	61.5±2.2 (21)	68.8±1.7 (19)	64.5±1.9 (23)	68.8±2.6 (19)	64.2±2.1 (22)	68.7±3.1 (12)	70.9±1.6 (35)	70.3±2.8 (16)	72.7±2.6 (20)	74±2.1 (27)	72.3±2.3 (18)	74.4±3.7 (5)	69±0.7 (237)
Mean ambulatory DBP (mmHg)	73.7±1.5 (16)	77.7±2 (13)	69.9±1.6 (19)	80.5±1.4 (13)	86.6±2.7 (17)	82.9±3.7 (7)	68.4±1.3 (26)	66±2.1 (11)	66.2±1.4 (18)	78.9±1.8 (20)	79.7±2.8 (14)	86.6±3.7 (2)	74.9±0.8 (176)
Mean ambulatory SBP (mmHg)	122.2±2.1 (16)	123.1±2 (13)	117.7±2.2 (19)	125.1±1.8 (13)	138.3±4 (17)	137±4.7 (7)	111.7±1.6 (26)	107.5±1.9 (11)	109±1.5 (18)	129±3.5 (20)	129.4±3.6 (14)	149.6±4.1 (2)	122±1.1 (176)
Mean ambulatory HR (mmHg)	68.7±2.7 (16)	73±2.4 (13)	70.2±2.8 (19)	76.9±3.6 (13)	75±2.6 (17)	77.4±4.3 (7)	75.1±1.5 (26)	74.2±2.2 (11)	75.5±2.2 (18)	79.6±2.2 (20)	79±2 (14)	82.6±8.6 (2)	75±0.8 (176)
Sleep DBP (mmHg)	68.5±1.9 (19)	68.3±2 (18)	66±1.8 (23)	72.9±2.2 (16)	75.7±2.5 (22)	72.5±3.3 (11)	61.4±1.4 (36)	59.6±2.5 (12)	62±1.9 (19)	70.4±1.9 (22)	68.9±2.7 (16)	79±6.2 (4)	67.6±0.7 (218)
Sleep SBP (mmHg)	114.8±2.2 (19)	112.1±1.8 (18)	111.8±2.1 (23)	120.1±3.7 (16)	124.9±3.9 (22)	122.2±4.3 (11)	102.1±1.5 (36)	99.2±2.2 (12)	103.4±2.5 (19)	117±3.9 (22)	115.1±3 (16)	134±10.9 (4)	112.7±1 (218)
Sleep HR (beast/min)	64.9±2.2 (18)	67.6±1.6 (18)	64.9±2.5 (23)	64.4±2.3 (16)	66±3.1 (22)	68.5±3.7 (11)	71.4±1.6 (36)	71.1±2.7 (12)	71.8±2.6 (19)	74.7±2.1 (21)	72.9±2.6 (16)	76.8±7 (4)	69.2±0.8 (216)
Sleep MAP (mmHg)	83.9±1.9 (19)	82.9±1.8 (18)	81.2±1.7 (23)	88.6±2.6 (16)	92.1±2.8 (22)	89.1±3.3 (11)	75±1.3 (36)	72.8±2.3 (12)	75.8±2 (19)	85.9±2.4 (22)	84.3±2.7 (16)	97.3±7.7 (4)	82.7±0.8 (218)
Sleep PP (mmHg)	46.3±1.6 (19)	43.8±1.5 (18)	45.9±1.5 (23)	47.2±2.1 (16)	49.2±2.7 (22)	49.6±3.2 (11)	40.8±1.1 (36)	39.6±0.9 (12)	41.4±1.5 (19)	46.6±2.9 (22)	46.2±1.4 (16)	55±5.1 (4)	45±0.6 (218)

Wake DBP (mmHg)	75.8±1.5 (20)	76.6±2 (18)	74.3±1.8 (23)	83.7±2.3 (16)	83.3±2.4 (22)	84.1±3.4 (11)	70.7±1.3 (35)	70±2.2 (12)	71.2±2.2 (19)	80.4±2 (21)	78±2.2 (16)	84.8±5.2 (4)	76.7±0.7 (217)
Wake SBP (mmHg)	125.3±2.3 (20)	124.2±2.2 (18)	124.1±2.2 (23)	132.5±3.3 (16)	137.6±3.9 (22)	138.3±4.8 (11)	114.9±1.6 (35)	111.2±1.7 (12)	117±3.2 (19)	130.7±4.1 (21)	128.6±3.2 (16)	145.8±10.6 (4)	125.5±1.1 (217)
Wake HR (beast/min)	74.6±2.9 (20)	79.8±1.7 (18)	75.6±2.5 (23)	77±3.1 (16)	81±3.7 (22)	81.6±3.6 (11)	80.1±1.4 (35)	79.6±2.3 (12)	84.3±2.3 (19)	84.8±2.6 (21)	82.6±2.7 (16)	85.3±5.2 (4)	80.1±0.8 (217)
Wake MAP (mmHg)	92.3±1.6 (20)	92.5±1.9 (18)	90.9±1.7 (23)	100±2.6 (16)	101.4±2.8 (22)	102.2±3.4 (11)	85.4±1.3 (35)	83.7±2 (12)	86.5±2.4 (19)	97.2±2.5 (21)	94.9±2.4 (16)	105.1±6.9 (4)	93±0.8 (217)
Wake PP (mmHg)	49.5±1.7 (20)	47.6±1.7 (18)	49.8±1.7 (23)	48.8±1.6 (16)	54.3±2.5 (22)	54.2±4 (11)	44.2±1.4 (35)	41.2±1.1 (12)	45.8±1.9 (19)	50.3±3 (21)	50.6±1.9 (16)	61±6.2 (4)	48.8±0.7 (217)
Overall PP (mmHg)	48.6±1.7 (16)	45.4±1 (13)	47.9±1.5 (19)	44.6±1.3 (13)	51.7±2.2 (17)	54.1±4.1 (7)	43.3±1.2 (26)	41.5±1.2 (11)	42.8±1.2 (18)	50.1±2.6 (20)	49.7±1.8 (14)	63±0.4 (2)	47.1±0.6 (176)

Table 4. Correlation[1] of obesity, stress and hemodynamic related traits with tobacco use

Trait	Current vs. never tobacco users		Current vs. former tobacco users		Former vs. never tobacco users	
	Z	P	Z	P	Z	P
BMI (kg/m2)	-3.3	**5.E-04**	-3.1	**1.E-03**	-0.8	2.E-01
Waist Hip ratio	-0.2	4.E-01	-1.1	1.E-01	0.5	3.E-01
Waist circumference (cm)	-2.4	**8.E-03**	-2.6	**4.E-03**	-0.3	4.E-01
Hip circumference (cm)	-3	**1.E-03**	-3	**1.E-03**	-0.6	3.E-01
Hip Thigh Proximal ratio	3.2	**6.E-04**	2	**3.E-02**	2.7	**4.E-03**
Thigh proximal circumference (cm)	-4.9	**5.E-07**	-3.6	**1.E-04**	-2.1	**2.E-02**
Thigh mid circumference (cm)	-3.7	**1.E-04**	-3.6	**1.E-04**	-1.4	9.E-02
Thigh distal circumference (cm)	-3.9	**5.E-05**	-2.3	**1.E-02**	-1.8	**3.E-02**
Skinfold bicep1 (mm)	-1.5	6.E-02	-0.8	2.E-01	-1.3	9.E-02
Skinfold bicep2 (mm)	-1.5	7.E-02	-0.9	2.E-01	-1.2	1.E-01

[1] Correlation test was performed using GEE method. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa. Correlation model is trait ~ sex + age + substance use.

Skinfold bicep3 (mm)	-1.6	6.E-02	-0.9	2.E-01	-1.3	1.E-01
Mean skinfold biceps (mm)	-1.5	7.E-02	-0.9	2.E-01	-1.2	1.E-01
Skinfold triceps1 (mm)	-1.9	**3.E-02**	-1	2.E-01	-0.9	2.E-01
Skinfold triceps2 (mm)	-1.8	**3.E-02**	-1	2.E-01	-0.8	2.E-01
Skinfold triceps3 (mm)	-1.6	6.E-02	-1.1	1.E-01	-0.7	2.E-01
Mean skinfold triceps (mm)	-1.9	**3.E-02**	-1.1	1.E-01	-0.8	2.E-01
Skinfold subscapular1 (mm)	-1.6	5.E-02	-0.8	2.E-01	-0.7	2.E-01
Skinfold subscapular2 (mm)	-1.4	8.E-02	-0.7	3.E-01	-0.6	3.E-01
Skinfold subscapular3 (mm)	-1.7	**4.E-02**	-0.8	2.E-01	-0.7	3.E-01
Mean skinfold subscapular (mm)	-1.6	5.E-02	-0.8	2.E-01	-0.7	3.E-01
Skinfold suprailiac1 (mm)	-2.3	**9.E-03**	-0.7	2.E-01	-1.6	6.E-02
Skinfold suprailiac2 (mm)	-2.3	**1.E-02**	-1.1	1.E-01	-1.3	1.E-01
Skinfold suprailiac3 (mm)	-2.2	**1.E-02**	-0.9	2.E-01	-1.2	1.E-01
Mean skinfold suprailiac (mm)	-2.3	**1.E-02**	-0.9	2.E-01	-1.4	8.E-02
Skinfold thigh1 (mm)	-2.2	**1.E-02**	-0.6	3.E-01	-1.7	**4.E-02**

Skinfold thigh2 (mm)	-2.1	**2.E-02**	-0.5	3.E-01	-1.9	**3.E-02**
Skinfold thigh3 (mm)	-2.3	**1.E-02**	-0.4	3.E-01	-2	**2.E-02**
Mean skinfold thigh (mm)	-2.3	**1.E-02**	-0.5	3.E-01	-1.9	**3.E-02**
Body fat percentage bioimpedance	-1.2	1.E-01	-0.4	4.E-01	-0.5	3.E-01
Body fat (%)	-2.5	**6.E-03**	-1.5	7.E-02	-0.9	2.E-01
Supine EP (pg/ml)	1.1	1.E-01	2.3	**1.E-02**	-0.9	2.E-01
Standing EP (pg/ml)	2.2	**1.E-02**	2.5	**6.E-03**	-0.3	4.E-01
Response EP (pg/ml)	1.4	8.E-02	0.9	2.E-01	0.3	4.E-01
Supine NE (pg/ml)	0.4	4.E-01	0.5	3.E-01	0	5.E-01
Standing NE (pg/ml)	0.8	2.E-01	1.2	1.E-01	-0.5	3.E-01
Response NE (pg/ml)	0.7	2.E-01	0.9	2.E-01	-0.6	3.E-01
DBP response to mental stress (mmHg)	0.3	4.E-01	1.4	7.E-02	0	5.E-01
DBP recovery mental stress (mmHg)	-0.2	4.E-01	1.4	7.E-02	-0.1	4.E-01
Delta DBP (mmHg)	-0.6	3.E-01	0.4	3.E-01	-0.3	4.E-01
SBP response to mental stress (mmHg)	-0.3	4.E-01	-1.4	8.E-02	1.3	1.E-01

SBP recovery from mental stress (mmHg)	-1.5	7.E-02	-1.8	**3.E-02**	0.9	2.E-01
Delta SBP (mmHg)	-2.1	**2.E-02**	-1.1	1.E-01	-0.6	3.E-01
HR response to mental stress (beast/min)	-1.8	**4.E-02**	-1.2	1.E-01	0.5	3.E-01
HR recovery from mental stress (beast/min)	-1.5	7.E-02	-1.9	**3.E-02**	0.9	2.E-01
Delta HR (beast/min)	0.3	4.E-01	-0.6	3.E-01	0.6	3.E-01
Average sitting DBP (mmHg)	-1.7	**4.E-02**	-2.4	**9.E-03**	0.9	2.E-01
Average sitting SBP (mmHg)	-3.6	**1.E-04**	-2.3	**1.E-02**	-0.5	3.E-01
Average sitting HR (beast/min)	0.4	3.E-01	0.5	3.E-01	-0.1	5.E-01
Mean ambulatory DBP (mmHg)	-2	**2.E-02**	-1.6	**5.E-02**	-0.1	5.E-01
Mean ambulatory SBP (mmHg)	-1	1.E-01	-1.5	7.E-02	0.2	4.E-01
Mean ambulatory HR (mmHg)	-0.6	3.E-01	0	5.E-01	-0.2	4.E-01
Sleep DBP (mmHg)	-0.7	3.E-01	0.5	3.E-01	-0.3	4.E-01
Sleep SBP (mmHg)	0	5.E-01	0.3	4.E-01	0	5.E-01
Sleep HR (beast/min)	0.3	4.E-01	0.1	5.E-01	0	5.E-01

Sleep MAP (mmHg)	-0.3	4.E-01	0.5	3.E-01	-0.2	4.E-01
Sleep PP (mmHg)	0.6	3.E-01	0.3	4.E-01	0	5.E-01
Wake DBP (mmHg)	-0.6	3.E-01	0	5.E-01	-0.5	3.E-01
Wake SBP (mmHg)	0.3	4.E-01	0.7	2.E-01	-0.1	5.E-01
Wake HR (beast/min)	1.7	**4.E-02**	0.1	4.E-01	0.5	3.E-01
Wake MAP (mmHg)	-0.2	4.E-01	0.3	4.E-01	-0.3	4.E-01
Wake PP (mmHg)	0.9	2.E-01	1	2.E-01	0.1	5.E-01
Overall PP (mmHg)	0.7	2.E-01	-0.4	4.E-01	0.6	3.E-01

Table 5. Correlation of obesity, stress and hemodynamic related traits with tobacco use in females and males

Trait	Females						Males					
	Current vs. never tobacco users		Current vs. former tobacco users		Former vs. never tobacco users		Current vs. never tobacco users		Current vs. former tobacco users		Former vs. never tobacco users	
	Z	P	Z	P	Z	P	Z	P	Z	P	Z	P
Hypertension status	-2.4	**8.E-03**	-3.0	**2.E-03**	0.9	2.E-01	-1.8	**4.E-02**	-2.0	**3.E-02**	-0.2	4.E-01
BMI (kg/m2)	0.0	5.E-01	-1.2	1.E-01	0.7	2.E-01	-4.1	**2.E-05**	-3.9	**5.E-05**	0.3	4.E-01
Waist Hip ratio	1.2	1.E-01	0.2	4.E-01	1.1	1.E-01	-2.0	**3.E-02**	-1.9	**3.E-02**	0.4	3.E-01
Waist circumference (cm)	0.0	5.E-01	-0.5	3.E-01	0.4	3.E-01	-3.4	**4.E-04**	-3.3	**5.E-04**	0.4	3.E-01
Hip circumference (cm)	-0.4	3.E-01	-0.7	2.E-01	0.2	4.E-01	-3.8	**8.E-05**	-4.1	**2.E-05**	0.4	3.E-01
Hip Thigh Proximal ratio	0.2	4.E-01	-0.5	3.E-01	1.0	2.E-01	3.9	**5.E-05**	3.5	**3.E-04**	1.0	1.E-01
Thigh proximal circumference (cm)	-0.9	2.E-01	-0.6	3.E-01	-0.3	4.E-01	-5.0	**2.E-07**	-4.4	**5.E-06**	-0.6	3.E-01
Thigh mid circumference (cm)	-0.8	2.E-01	-1.3	1.E-01	0.3	4.E-01	-3.4	**3.E-04**	-3.9	**5.E-05**	0.1	5.E-01
Thigh distal circumference (cm)	-1.2	1.E-01	-0.6	3.E-01	-0.4	3.E-01	-4.2	**1.E-05**	-3.0	**2.E-03**	-0.2	4.E-01
Skinfold bicep1 (mm)	1.2	1.E-01	0.5	3.E-01	0.5	3.E-01	-2.2	**1.E-02**	-0.8	2.E-01	-0.6	3.E-01

Skinfold bicep2 (mm)	1.2	1.E-01	0.2	4.E-01	0.7	2.E-01	-2.3	**1.E-02**	-0.7	2.E-01	-0.8	2.E-01
Skinfold bicep3 (mm)	1.0	1.E-01	0.2	4.E-01	0.7	2.E-01	-2.4	**7.E-03**	-0.7	2.E-01	-0.9	2.E-01
Mean skinfold biceps (mm)	1.1	1.E-01	0.3	4.E-01	0.7	3.E-01	-2.3	**1.E-02**	-0.7	2.E-01	-0.8	2.E-01
Skinfold triceps1 (mm)	0.9	2.E-01	0.2	4.E-01	0.6	3.E-01	-2.6	**5.E-03**	-1.2	1.E-01	0.1	5.E-01
Skinfold triceps2 (mm)	0.8	2.E-01	0.1	5.E-01	0.8	2.E-01	-2.3	**1.E-02**	-1.0	2.E-01	-0.2	4.E-01
Skinfold triceps3 (mm)	1.2	1.E-01	0.1	5.E-01	1.2	1.E-01	-2.3	**1.E-02**	-1.1	1.E-01	-0.3	4.E-01
Mean skinfold triceps (mm)	0.9	2.E-01	0.1	5.E-01	0.7	2.E-01	-2.4	**8.E-03**	-1.2	1.E-01	0.0	5.E-01
Skinfold subscapular1 (mm)	2.3	**1.E-02**	1.0	1.E-01	1.7	**5.E-02**	-3.7	**1.E-04**	-2.1	**2.E-02**	-1.5	7.E-02
Skinfold subscapular2 (mm)	2.4	**7.E-03**	1.2	1.E-01	1.7	**5.E-02**	-3.8	**8.E-05**	-2.2	**1.E-02**	-1.4	8.E-02
Skinfold subscapular3 (mm)	2.3	**1.E-02**	1.0	2.E-01	1.7	**4.E-02**	-4.2	**2.E-05**	-2.2	**1.E-02**	-1.4	8.E-02
Mean skinfold subscapular (mm)	2.3	**1.E-02**	1.1	1.E-01	1.7	**5.E-02**	-3.9	**5.E-05**	-2.2	**1.E-02**	-1.5	7.E-02
Skinfold suprailiac1 (mm)	1.0	2.E-01	0.8	2.E-01	1.2	1.E-01	-3.7	**1.E-04**	-1.9	**3.E-02**	-1.0	2.E-01
Skinfold suprailiac2 (mm)	0.9	2.E-01	0.4	3.E-01	1.6	5.E-02	-3.4	**3.E-04**	-2.1	**2.E-02**	-0.7	2.E-01
Skinfold suprailiac3 (mm)	1.1	1.E-01	0.7	2.E-01	1.6	5.E-02	-3.3	**4.E-04**	-2.1	**2.E-02**	-0.8	2.E-01
Mean skinfold suprailiac (mm)	1.0	2.E-01	0.7	3.E-01	1.5	7.E-02	-3.5	**2.E-04**	-2.0	**2.E-02**	-0.9	2.E-01

Skinfold thigh1 (mm)	0.3	4.E-01	0.6	3.E-01	0.2	4.E-01	-2.6	**5.E-03**	-0.9	2.E-01	-0.4	3.E-01
Skinfold thigh2 (mm)	0.2	4.E-01	0.5	3.E-01	0.1	5.E-01	-2.6	**4.E-03**	-0.9	2.E-01	-0.7	2.E-01
Skinfold thigh3 (mm)	-0.1	4.E-01	0.5	3.E-01	0.0	5.E-01	-2.7	**3.E-03**	-0.8	2.E-01	-0.7	3.E-01
Mean skinfold thigh (mm)	0.2	4.E-01	0.7	2.E-01	0.1	5.E-01	-2.6	**4.E-03**	-0.9	2.E-01	-0.5	3.E-01
Body fat percentage bioimpedance	0.8	2.E-01	0.7	2.E-01	1.0	2.E-01	-2.1	**2.E-02**	-1.1	1.E-01	-0.3	4.E-01
Body fat (%)	1.1	1.E-01	0.4	3.E-01	1.6	6.E-02	-4.9	**4.E-07**	-2.7	**4.E-03**	-1.1	1.E-01
Supine EP (pg/ml)	1.1	1.E-01	1.2	1.E-01	-0.4	4.E-01	0.6	3.E-01	1.6	6.E-02	-0.5	3.E-01
Standing EP (pg/ml)	2.7	**3.E-03**	3.3	**6.E-04**	-0.5	3.E-01	0.3	4.E-01	1.1	1.E-01	-0.1	5.E-01
Response EP (pg/ml)	1.9	**3.E-02**	1.8	**4.E-02**	-0.3	4.E-01	0.2	4.E-01	-0.1	5.E-01	0.1	5.E-01
Supine NE (pg/ml)	0.4	3.E-01	-0.2	4.E-01	0.7	2.E-01	0.4	3.E-01	0.8	2.E-01	0.0	5.E-01
Standing NE (pg/ml)	0.0	5.E-01	0.4	4.E-01	-0.1	4.E-01	1.2	1.E-01	1.1	1.E-01	-0.3	4.E-01
Response NE (pg/ml)	-0.3	4.E-01	0.6	3.E-01	-0.5	3.E-01	1.1	1.E-01	0.8	2.E-01	-0.5	3.E-01
DBP recovery mental stress (mmHg)	-0.7	2.E-01	-1.0	2.E-01	2.4	**9.E-03**	0.5	3.E-01	2.7	**3.E-03**	-0.7	3.E-01
DBP response to mental stress (mmHg)	-0.4	3.E-01	-1.0	2.E-01	-0.4	3.E-01	1.0	2.E-01	2.2	**1.E-02**	0.2	4.E-01

Delta DBP (mmHg)	-0.4	3.E-01	-0.4	3.E-01	0.5	3.E-01	-0.4	4.E-01	0.6	3.E-01	-0.6	3.E-01
SBP recovery from mental stress (mmHg)	-1.9	**3.E-02**	-1.7	**5.E-02**	-2.3	**1.E-02**	-0.3	4.E-01	-0.9	2.E-01	1.7	**4.E-02**
SBP response to mental stress (mmHg)	-1.3	9.E-02	-1.8	**3.E-02**	-1.4	7.E-02	0.5	3.E-01	-0.5	3.E-01	2.2	**2.E-02**
Delta SBP (mmHg)	-2.0	**3.E-02**	-0.4	3.E-01	-0.3	4.E-01	-1.2	1.E-01	-1.2	1.E-01	-0.3	4.E-01
HR recovery from mental stress (beast/min)	-0.6	3.E-01	-1.4	9.E-02	0.3	4.E-01	-0.5	3.E-01	-0.9	2.E-01	1.5	7.E-02
HR response to mental stress (beast/min)	-0.3	4.E-01	-1.1	1.E-01	0.4	3.E-01	-1.0	2.E-01	-0.4	4.E-01	2.7	**4.E-03**
Delta HR (beast/min)	-1.3	9.E-02	0.0	5.E-01	-0.6	3.E-01	1.1	1.E-01	-0.4	4.E-01	1.1	1.E-01
Average sitting DBP (mmHg)	-1.7	**4.E-02**	-3.3	**5.E-04**	0.9	2.E-01	-1.5	7.E-02	-1.5	6.E-02	-0.2	4.E-01
Average sitting SBP (mmHg)	-3.7	**9.E-05**	-2.6	**5.E-03**	-0.5	3.E-01	-1.8	**4.E-02**	-1.4	8.E-02	-0.3	4.E-01
Average sitting HR (beast/min)	0.3	4.E-01	0.4	4.E-01	0.2	4.E-01	0.2	4.E-01	-0.1	5.E-01	-0.1	5.E-01
Mean ambulatory DBP (mmHg)	-0.9	2.E-01	-0.1	4.E-01	-1.8	**4.E-02**	-1.9	**3.E-02**	-3.4	**4.E-04**	1.4	8.E-02
Mean ambulatory SBP (mmHg)	-0.8	2.E-01	-0.2	4.E-01	-1.1	1.E-01	-0.4	4.E-01	-2.1	**2.E-02**	1.9	**3.E-02**
Mean ambulatory HR (mmHg)	0.0	5.E-01	-0.2	4.E-01	-0.1	5.E-01	-2.9	**2.E-03**	-0.3	4.E-01	-0.5	3.E-01

Sleep DBP (mmHg)	0.5	3.E-01	0.7	2.E-01	-0.7	2.E-01	-1.3	1.E-01	-0.5	3.E-01	0.2	4.E-01
Sleep SBP (mmHg)	0.7	2.E-01	0.9	2.E-01	-0.5	3.E-01	-0.5	3.E-01	-0.7	3.E-01	0.4	4.E-01
Sleep HR (beast/min)	0.1	5.E-01	1.1	1.E-01	-0.3	4.E-01	0.5	3.E-01	0.0	5.E-01	0.0	5.E-01
Sleep MAP (mmHg)	0.7	3.E-01	0.8	2.E-01	-0.8	2.E-01	-0.9	2.E-01	-0.5	3.E-01	0.3	4.E-01
Sleep PP (mmHg)	0.9	2.E-01	0.6	3.E-01	0.5	3.E-01	0.3	4.E-01	-0.1	5.E-01	0.2	4.E-01
Wake DBP (mmHg)	0.4	3.E-01	-0.1	5.E-01	-0.8	2.E-01	-1.0	2.E-01	-0.2	4.E-01	-0.3	4.E-01
Wake SBP (mmHg)	0.7	3.E-01	1.1	1.E-01	-0.7	2.E-01	0.0	5.E-01	-0.2	4.E-01	0.7	2.E-01
Wake HR (beast/min)	2.9	**2.E-03**	1.8	**4.E-02**	-0.1	5.E-01	0.3	4.E-01	-0.6	3.E-01	0.5	3.E-01
Wake MAP (mmHg)	0.4	3.E-01	0.1	5.E-01	-0.9	2.E-01	-0.5	3.E-01	-0.3	4.E-01	0.2	4.E-01
Wake PP (mmHg)	0.9	2.E-01	1.8	**4.E-02**	-0.5	3.E-01	0.6	3.E-01	0.0	5.E-01	1.0	2.E-01
Overall PP (mmHg)	0.0	5.E-01	-0.4	4.E-01	-0.1	5.E-01	1.9	**3.E-02**	-0.2	4.E-01	1.5	7.E-02

Table 6. Correlation of obesity, stress and hemodynamic related traits with tobacco use in hypertensives and normotensives

Trait	Hypertensives						Normotensives					
	Current vs. never tobacco users		Current vs. former tobacco users		Former vs. never tobacco users		Current vs. never tobacco users		Current vs. former tobacco users		Former vs. never tobacco users	
	Z	P	Z	P	Z	P	Z	P	Z	P	Z	P
BMI (kg/m2)	-2.3	**1.E-02**	-1.9	**3.E-02**	0.0	5.E-01	-1.8	**4.E-02**	-1.4	9.E-02	-1.4	8.E-02
Waist Hip ratio	-0.4	3.E-01	-1.2	1.E-01	0.8	2.E-01	0.6	3.E-01	0.2	4.E-01	-0.2	4.E-01
Waist circumference (cm)	-1.4	7.E-02	-1.1	1.E-01	0.0	5.E-01	-1.5	6.E-02	-1.4	8.E-02	-1.0	2.E-01
Hip circumference (cm)	-2.2	**1.E-02**	-1.0	2.E-01	-0.5	3.E-01	-2.3	**1.E-02**	-2.6	**4.E-03**	-0.7	2.E-01
Hip Thigh Proximal ratio	2.9	**2.E-03**	1.6	6.E-02	2.4	**8.E-03**	2.7	**3.E-03**	0.7	2.E-01	1.9	**3.E-02**
Thigh proximal circumference (cm)	-2.9	**2.E-03**	-1.7	**4.E-02**	-1.6	6.E-02	-3.7	**9.E-05**	-2.4	**8.E-03**	-1.6	6.E-02
Thigh mid circumference (cm)	-2.6	**5.E-03**	-2.0	**2.E-02**	-1.1	1.E-01	-1.4	8.E-02	-1.9	**3.E-02**	-0.7	3.E-01
Thigh distal circumference (cm)	-2.7	**3.E-03**	-0.9	2.E-01	-1.4	8.E-02	-2.7	**3.E-03**	-2.3	**1.E-02**	-1.5	7.E-02
Skinfold bicep1 (mm)	-1.0	2.E-01	-0.3	4.E-01	-1.0	2.E-01	0.0	5.E-01	-0.3	4.E-01	-0.4	3.E-01
Skinfold bicep2 (mm)	-0.8	2.E-01	-0.4	4.E-01	-0.5	3.E-01	-0.1	4.E-01	-0.1	5.E-01	-0.5	3.E-01
Skinfold bicep3 (mm)	-0.8	2.E-01	-0.2	4.E-01	-0.7	2.E-01	-0.4	4.E-01	-0.3	4.E-01	-0.5	3.E-01

Mean skinfold biceps (mm)	-0.9	2.E-01	-0.3	4.E-01	-0.7	2.E-01	-0.2	4.E-01	-0.3	4.E-01	-0.4	3.E-01
Skinfold triceps1 (mm)	-0.5	3.E-01	0.3	4.E-01	-1.0	2.E-01	-1.2	1.E-01	-1.0	1.E-01	0.0	5.E-01
Skinfold triceps2 (mm)	-0.5	3.E-01	0.2	4.E-01	-0.9	2.E-01	-1.1	1.E-01	-1.0	2.E-01	0.0	5.E-01
Skinfold triceps3 (mm)	-0.4	3.E-01	0.1	4.E-01	-0.7	2.E-01	-1.0	2.E-01	-1.0	1.E-01	0.1	4.E-01
Mean skinfold triceps (mm)	-0.5	3.E-01	0.1	4.E-01	-0.8	2.E-01	-1.1	1.E-01	-1.0	2.E-01	0.0	5.E-01
Skinfold subscapular1 (mm)	-1.4	9.E-02	-0.6	3.E-01	0.6	3.E-01	-1.0	2.E-01	0.1	5.E-01	-2.3	**1.E-02**
Skinfold subscapular2 (mm)	-1.0	2.E-01	-0.5	3.E-01	0.7	2.E-01	-0.9	2.E-01	0.0	5.E-01	-2.2	**1.E-02**
Skinfold subscapular3 (mm)	-1.3	1.E-01	-0.6	3.E-01	0.6	3.E-01	-1.1	1.E-01	-0.1	5.E-01	-2.3	**1.E-02**
Mean skinfold subscapular (mm)	-1.2	1.E-01	-0.6	3.E-01	0.6	3.E-01	-1.0	1.E-01	0.0	5.E-01	-2.3	**1.E-02**
Skinfold suprailiac1 (mm)	-0.6	3.E-01	0.5	3.E-01	-0.1	5.E-01	-2.8	**3.E-03**	-0.6	3.E-01	-2.8	**3.E-03**
Skinfold suprailiac2 (mm)	-0.7	3.E-01	0.3	4.E-01	0.0	5.E-01	-2.7	**4.E-03**	-1.0	2.E-01	-2.5	**7.E-03**
Skinfold suprailiac3 (mm)	-0.7	2.E-01	0.3	4.E-01	0.0	5.E-01	-2.4	**8.E-03**	-0.8	2.E-01	-2.3	**1.E-02**
Mean skinfold suprailiac (mm)	-0.7	3.E-01	0.4	3.E-01	-0.1	5.E-01	-2.7	**4.E-03**	-0.8	2.E-01	-2.6	**5.E-03**
Skinfold thigh1 (mm)	-0.6	3.E-01	0.8	2.E-01	-1.3	9.E-02	-2.0	**2.E-02**	-1.7	**5.E-02**	-0.4	3.E-01
Skinfold thigh2 (mm)	-0.7	3.E-01	0.8	2.E-01	-1.4	9.E-02	-1.9	**3.E-02**	-1.6	6.E-02	-0.7	2.E-01
Skinfold thigh3 (mm)	-0.8	2.E-01	0.7	3.E-01	-1.4	8.E-02	-2.0	**2.E-02**	-1.4	9.E-02	-0.9	2.E-01

Mean skinfold thigh (mm)	-0.7	2.E-01	0.7	2.E-01	-1.3	9.E-02	-2.0	**2.E-02**	-1.5	6.E-02	-0.6	3.E-01
Body fat percentage bioimpedance	0.0	5.E-01	3.1	**8.E-04**	-0.8	2.E-01	-1.3	1.E-01	-1.7	**4.E-02**	0.3	4.E-01
Body fat (%)	-1.2	1.E-01	-0.5	3.E-01	-0.1	5.E-01	-2.3	**1.E-02**	-1.0	2.E-01	-1.7	**5.E-02**
Supine EP (pg/ml)	0.5	3.E-01	1.2	1.E-01	0.0	5.E-01	0.3	4.E-01	2.2	**1.E-02**	-1.5	6.E-02
Standing EP (pg/ml)	0.9	2.E-01	0.1	4.E-01	0.5	3.E-01	1.6	5.E-02	3.6	**2.E-04**	-1.2	1.E-01
Response EP (pg/ml)	-0.8	2.E-01	-0.7	2.E-01	0.7	3.E-01	1.9	**3.E-02**	2.4	**9.E-03**	-0.2	4.E-01
Supine NE (pg/ml)	0.6	3.E-01	0.2	4.E-01	-0.2	4.E-01	0.4	3.E-01	0.3	4.E-01	1.1	1.E-01
Standing NE (pg/ml)	0.9	2.E-01	0.5	3.E-01	-0.7	2.E-01	0.8	2.E-01	0.7	2.E-01	0.6	3.E-01
Response NE (pg/ml)	0.5	3.E-01	1.1	1.E-01	-1.1	1.E-01	0.8	2.E-01	0.4	4.E-01	0.5	3.E-01
DBP recovery mental stress (mmHg)	0.8	2.E-01	1.8	**4.E-02**	0.0	5.E-01	0.0	5.E-01	1.1	1.E-01	-0.1	5.E-01
DBP response to mental stress (mmHg)	1.0	2.E-01	1.7	**4.E-02**	0.1	5.E-01	0.4	3.E-01	0.7	2.E-01	0.6	3.E-01
Delta DBP (mmHg)	-0.3	4.E-01	-0.6	3.E-01	0.5	3.E-01	-0.7	2.E-01	1.2	1.E-01	-1.1	1.E-01
SBP recovery from mental stress (mmHg)	-0.6	3.E-01	-1.1	1.E-01	0.8	2.E-01	-0.1	4.E-01	-0.3	4.E-01	0.7	2.E-01
SBP response to mental stress (mmHg)	0.2	4.E-01	-0.1	5.E-01	0.9	2.E-01	0.6	3.E-01	-0.3	4.E-01	1.8	**4.E-02**
Delta SBP (mmHg)	-0.9	2.E-01	-0.7	3.E-01	0.0	5.E-01	-2.2	**1.E-02**	0.0	5.E-01	-1.5	6.E-02
HR recovery from mental stress (beast/min)	-0.4	4.E-01	-0.6	3.E-01	-0.2	4.E-01	-1.0	2.E-01	-1.6	6.E-02	0.9	2.E-01

HR response to mental stress (beast/min)	-0.4	3.E-01	0.3	4.E-01	-0.2	4.E-01	-1.5	7.E-02	-1.0	1.E-01	0.5	3.E-01
Delta HR (beast/min)	-0.4	3.E-01	-0.9	2.E-01	-0.5	3.E-01	0.4	3.E-01	-0.5	3.E-01	1.2	1.E-01
Average sitting DBP (mmHg)	-0.5	3.E-01	-0.5	3.E-01	0.8	2.E-01	-1.6	6.E-02	-2.5	**7.E-03**	0.7	2.E-01
Average sitting SBP (mmHg)	0.3	4.E-01	0.2	4.E-01	-0.3	4.E-01	-3.9	**4.E-05**	-1.7	**5.E-02**	-1.2	1.E-01
Average sitting HR (beast/min)	0.4	3.E-01	1.9	**3.E-02**	-1.6	5.E-02	1.0	2.E-01	-0.5	3.E-01	1.8	**4.E-02**
Mean ambulatory DBP (mmHg)	1.6	6.E-02	-0.4	4.E-01	0.9	2.E-01	-2.1	**2.E-02**	-1.2	1.E-01	-0.1	5.E-01
Mean ambulatory SBP (mmHg)	4.0	**4.E-05**	1.5	7.E-02	0.8	2.E-01	-1.8	**4.E-02**	-0.6	3.E-01	-1.0	2.E-01
Mean ambulatory HR (mmHg)	0.7	2.E-01	0.5	3.E-01	0.3	4.E-01	-0.3	4.E-01	-0.2	4.E-01	-0.1	4.E-01
Sleep DBP (mmHg)	0.9	2.E-01	2.8	**3.E-03**	-0.2	4.E-01	-0.7	2.E-01	1.0	2.E-01	-0.4	3.E-01
Sleep SBP (mmHg)	1.8	**4.E-02**	2.3	**1.E-02**	0.2	4.E-01	0.0	5.E-01	1.1	1.E-01	-0.8	2.E-01
Sleep HR (beast/min)	1.1	1.E-01	1.6	6.E-02	-0.3	4.E-01	-0.5	3.E-01	-0.3	4.E-01	-0.6	3.E-01
Sleep MAP (mmHg)	1.4	8.E-02	2.6	**5.E-03**	-0.1	5.E-01	-0.3	4.E-01	1.0	1.E-01	-0.6	3.E-01
Sleep PP (mmHg)	1.6	5.E-02	1.1	1.E-01	0.3	4.E-01	0.7	3.E-01	1.2	1.E-01	-1.3	1.E-01
Wake DBP (mmHg)	1.1	1.E-01	1.6	5.E-02	-0.5	3.E-01	-0.4	4.E-01	0.0	5.E-01	0.5	3.E-01
Wake SBP (mmHg)	2.7	**3.E-03**	2.8	**2.E-03**	-0.1	5.E-01	-0.1	5.E-01	1.3	9.E-02	-1.1	1.E-01
Wake HR (beast/min)	1.8	**3.E-02**	4.3	**1.E-05**	-0.2	4.E-01	1.1	1.E-01	-0.2	4.E-01	1.1	1.E-01

Wake MAP (mmHg)	2.0	**2.E-02**	1.9	**3.E-02**	-0.4	3.E-01	-0.6	3.E-01	0.4	3.E-01	-0.1	5.E-01
Wake PP (mmHg)	3.7	**9.E-05**	0.5	3.E-01	0.7	3.E-01	0.2	4.E-01	1.7	**5.E-02**	-0.9	2.E-01
Overall PP (mmHg)	4.7	**1.E-06**	2.7	**4.E-03**	1.0	2.E-01	-0.5	3.E-01	1.0	2.E-01	-1.8	**3.E-02**

Table 7. Significantly shared SNPs between tobacco use and correlated obesity, stress and hemodynamic related traits

Status	Trait	SNP	Univariate P	Gene	Combined P
General	Waist circumference	rs679783	7.40E-05	AGBL4	2.30E-07
	Former vs. never tobacco users	rs679783	1.60E-04		
	Current vs. never tobacco users	rs679783	6.00E-04		
	Hip circumference	rs679783	8.40E-04		
	Thigh mid circumference	rs679783	1.90E-03		
	Thigh distal circumference	rs679783	2.50E-03		
	BMI	rs679783	2.50E-03		
	Thigh proximal circumference	rs679783	3.30E-03		
Males	Former vs. never tobacco users	rs947084	2.20E-05	OBSCN	1.20E-06
	Current vs. former tobacco users	rs947084	5.10E-04		
	Hip Thigh Proximal ratio	rs947084	3.20E-03		
Hypertensives	Current vs. former tobacco users	rs36950	2.70E-05	KCNN2	1.54E-08
	Current vs. never tobacco users	rs36950	2.70E-05		
	SBP recovery from mental stress	rs36950	1.30E-03		
	Mean ambulatory SBP	rs36950	8.80E-03		

Table 8. Bivariate association analysis on significantly shared SNPs

Status	Tobacco use	Correlated trait	SNP	Bivariate P	Gene
General	Former vs. never tobacco users	Hip circumference	rs679783	1.2E-03	AGBL4
	Current vs. never tobacco users	Hip circumference	rs679783	1.9E-04	
	Former vs. never tobacco users	Thigh mid circumference	rs679783	9.2E-04	
	Current vs. never tobacco users	Thigh mid circumference	rs679783	7.3E-04	
	Former vs. never tobacco users	Thigh distal circumference	rs679783	1.5E-03	
	Current vs. never tobacco users	Thigh distal circumference	rs679783	1.3E-03	
	Former vs. never tobacco users	BMI	rs679783	2.2E-03	
	Current vs. never tobacco users	BMI	rs679783	8.0E-04	
	Former vs. never tobacco users	Thigh proximal circumference	rs679783	2.7E-03	
	Current vs. never tobacco users	Thigh proximal circumference	rs679783	1.0E-03	
	Former vs. never tobacco users	Waist circumference	rs679783	1.2E-04	
	Current vs. never tobacco users	Waist circumference	rs679783	3.0E-05	
Males	Former vs. never tobacco users	Hip Thigh Proximal ratio	rs947084	5.2E-06	OBSCN
	Current vs. former tobacco users	Hip Thigh Proximal ratio	rs947084	3.9E-03	
Hypertensives	Current vs. never tobacco users	SBP recovery from mental stress	rs36956	2.4E-06	KCNN2
	Current vs. former tobacco users	SBP recovery from mental stress	rs36956	2.4E-06	
	Current vs. former tobacco users	SBP recovery from mental stress	rs36950	8.1E-06	
	Current vs. never tobacco users	SBP recovery from mental stress	rs36950	8.1E-06	
	Current vs. former tobacco users	Mean ambulatory SBP	rs36950	2.5E-05	
	Current vs. never tobacco users	Mean ambulatory SBP	rs36950	2.5E-05	

Discussion

4.1 Shared genetic factors among polygenic disorders

Earlier large-scale clinical studies indicated that many patients suffer from several complex disorders simultaneously. For instance, metabolic syndrome is characterized by a group of risk factors including central obesity, dyslipidemia, hypertension, and impaired glucose/insulin homeostasis. Among patients diagnosed with cardiovascular disorders, only 20–25% of patients have only one concomitant disease; while, the remainders suffer from two or more disorders. It is also reported that 40% of patients with type 2 diabetes have three or more concomitant diseases.[1-4]

Growing number of studies pointed that apart from environmental factors genetic determinants are also involved in these phenotypic correlations. Williams et al[5] investigated the correlation between hypertension, migraine, Raynaud's phenomenon and coronary artery disease in a sample of 2204 individuals that included 525 monozygous twins and 577 dizygous twins and calculated genetic correlations among these traits, they reported significant genetic contribution to all four traits with heritabilities ranging from 0.34 to 0.64 and found that a shared genetic componet explains the phenotypic correlations among these vascular conditions.

Rzhetzky et al[6] analyzed 1.5 million medical records involving 161 diseases represent a broad spectrum of disorders affecting diverse physiological systems and estimate pairwise correlations of disease co-occurrences as well as the extent of genetic

overlap among them. They noted that disease phenotypes form a highly connected network of strong pairwise correlations and found that multifactorial disease phenotypes are highly genetically correlated traits and recommend the design of gene-mapping studies that analyze multiple disorders jointly.

In another study using data from Mendelian Inheritance in Man (OMIM) database that represents and up-to-date repository of all known disease genes and the disorders, Goh et al[7] constructed a network of human diseases in which two disorders are connected to each other if they share at least one gene. Although they found clustering among the disease of the same class but the resulted network did not fall into many single nodes corresponding to specific disorders or grouped into small clusters of a few closely related disorders; instead it form a large network of human diseases. Of 1,284 disorders in OMIM database, 867 had at least one link to other disorders, and 516 disorders form a giant component, suggesting that the genetic origins of most diseases, to some extent, are shared with other diseases.

In a more recent study, Torkamani et al[8] compared the results of GWAS of bipolar disorder, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, type 1 diabetes, and type 2 diabetes performed in Wellcome Trust Case Control Consortium (WTCCC) GWAS dataset. They found a large number of significantly shared SNPs not only among the related ones but also among seemingly unrelated traits e.g. among bipolar disorder with the metabolic disorders, coronary artery disease and type 2 diabetes or between hypertension and Crohn's disease.

Despite numerous evidences suggest pleiotropic genes are not rare among polygenic disorders, current genome-wide scan studies are normally analyze different phenotypes in isolation and ignores genes that are showing pleiotropic effect and important to the pathogenesis of correlated disorders and can provide several benefits.[7; 9-11]

Motivated by these lines of evidences, we aimed to investigate for shared genomic factors of habitual alcohol, tobacco and coffee use, response to mental and physical stress, obesity-related anthropometric traits and heart rate (HR) and blood pressure (BP)

measurements. Alcohol, tobacco and coffee are the most commonly consumed psychoactive substances in the world. Their concurrent use has been consistently shown across a wide variety of populations.[15] Stress has been found both in experimental and clinical research to relate to initiation, intensification and relapse to substance use;[16] besides, both stress and substance use are considered as contributing factors to development of cardiovascular disease and cardiovascular risk factors including obesity.[12-14; 59]

HPA axis, the main component of body's response to stressors has been implicated in substance use, obesity and regulation of cardiovascular system;[17-21] moreover, number of genes involved in HPA axis were found to connect these traits; for instance, variations in mu opioid receptor (OPRM1) have been reported to influence response to stressors, food intake and substance use;[12; 17; 21] nonetheless, similar to other polygenic traits, substance use, obesity, stress, and cardiovascular traits have multifactorial etiology with a substantial complex genetic components and further studies are required to uncover the genetic nature and relations among these traits; therefore, we hypothesized that the links among substance use habits, obesity, stress, and related cardiovascular outcomes may be in part due to variations in shared genetic factors.

In this study consistent with the observed phenotyping correlations among these traits. Following genome wide scans, we found numerous significantly shared SNPs among these traits which further support the hypothesis indicates phenotypic correlation is a predictor of genotypic overlaps in polygenic disorders.

4.2 Synaptic plasticity

The identified shared loci were not unrelated; in contrast, their products interact with one another through protein–protein interactions, display higher expression and similar expression profiles in brain-related tissues and finally cluster in synaptic plasticity related pathways. Together, these findings suggest that synaptic plasticity, an important

foundation of learning and memory is a common interface behind substance use, stress, obesity, HR and BP.

This is consistent with previous implications of synaptic plasticity for these traits. For instance, physiological studies have also shown that both substance use and stress influence the synaptic plasticity and substance use can change the sensitivity of synaptic plasticity to stressors;[22; 23] moreover, an influential hypothesis suggests that addiction is a strong form of learning. Synaptic plasticity also appears to be involved in the regulation of energy homeostasis and is acting as an important path through which peripheral metabolic hormones influence brain functions.[24] In fact, similar to psychoactive drugs, palatable foods can activate the brain reward system and pharmacological blockade of, or experimental damage to forebrain dopamine systems attenuates free feeding and lever-pressing for food reward, as well as the rewarding effects of psychoactive substances;[25; 26] moreover, synaptic sensitization, an attribute of thalamocortical and memory neurons, is responsible for hypothalamic hyperresponsiveness to environmental stimuli, it ensures that repeated stimulation of the defense pathway makes it respond to ever milder stresses, so that hypertension eventually becomes permanent.[59]

We assume the interaction between genetic make-up of synapses with environmental factors including stress influence individuals's life styles and habits; in the other side, the taken habits and lifestyle influence the body's systems including cardiovascular system and modify the cardiovascular outcomes as well as cardiovascular risk factors including body weight; nonetheless, similar to different routes ended to the same location, there are other factors that influence the cardiovascular system through other mechanisms. Our results also suggest that synaptic plasticity may be a common interface behind many of other complex disorders in which life style is a contributing factor.[6; 7; 27]

4.3 Network thinking

The observation that identified shared genes among substance use, obesity, stress and hemodynamic traits tend to cluster and share interaction and function in synaptic processes indicates that these genes rather than being unrelated, are components of a functional module. Consistent with our findings, by comparing GWAS results of bipolar disorder, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, type 1 diabetes, and type 2 diabetes performed in WTCCC dataset, Torkamnai et al[8] found set of signaling factors including G-protein, adenylate cyclase, protein kinase A and C, inositol trisphosphate (IP3), and calcium signaling mechanisms are behind general morbidity of these traits; therefore, considering network-based approaches can help elucidate common biological interfaces underlie comorbidity of disorders

Our findings also support network-based model proposed for polygenic disorders which indicates cellular networks are modular, consisting of groups of highly interconnected proteins responsible for specific cellular functions and a disorder represents the perturbation or breakdown of a specific functional module caused by variations in components of the network producing recognizable developmental and/or physiological abnormalities.

4.4 Epistasis and pleiotropy

Such Network-based view also leads to the conclusion that unlike Mendelian disorders, epistasis and pleiotropy are not rare occurrences, but ubiquitous and inherent genetic properties of polygenic disorders. Pleiotropy, in which one mutation causes multiple phenotypes, has traditionally been seen as a deviation from the traditional observation in which one gene affects one phenotype. Epistasis, or gene–gene interaction, has also been treated as an exception to the Mendelian one gene–one phenotype paradigm; however, assuming dysregulation of a modular network is behind a polygenic disorder and considering the widespread connectivity of networks; therefore, epistasis and pleiotropy are

expected byproducts of bimolecular networks; hence, these phenomena should not be ignored or treated as rare occurrences in genetic analysis of complex disorders, but rather as features that can help to elucidate the genetic nature and underlying mechanisms.[28]

4.5 Sex and hypertension differences

We found that large portions of variations of studied traits are attributed to sex and hypertension status, focusing on tobacco use we noted that even degree and the direction of correlations of obesity, hemodynamic and stress related traits with tobacco use vary according to sex and hypertension status; for instance, while in males, analysis of both global and regional measurements of obesity indicated that current tobacco users are less obese compared to never or former tobacco users, obesity related measures were not significantly different among tobacco use statues in females and even, unlike males, subscapular skinfold measurement was higher in female smokers compared to non smokers.

Consistent with above findings, subgroup analysis revealed shared loci among substance use, obesity, stress and hemodynamic traits; moreover, we also found sex- and hypertension differences in heritabilities of many of these traits.[29; 30] Detection of such specific loci may be due to their sex- and hypertension-specific effects or co-segregation with epistatic sex- and hypertension specific genetic factors that modify their effects;[29-32] therefore, adjustment for sex and hypertension status can obscure identification of such variations.

Hypertension-specific analysis uncovered SNP, rs4687150 inside IL1RAP gene which was negatively associated ($P = 0.0004$) to coffee use in hypertensives and positively associated ($P = 0.0002$) in normotensives. Sex specific genetic analysis revealed SNPs, rs4888197 and rs847936 inside PLCG2 and near SCIN which were negatively associated ($P < 0.001$) in females and positively associated ($P < 0.001$) in males. All three genes, IL1RAP, PLCG2, and SCIN shared synaptic function and consistent with the finding of SCIN gene for tobacco use, it is reported that nicotinic-receptor stimulation induces the

intracellular redistribution of SCIN protein;[33] therefore, it is obvious that mixing the two groups together as it is carried out in general analysis will eliminate such association signals.

Identification of hypertension specific loci also indicates that there are genetic links between these traits and hypertension which further support the phenotypic relatedness of substance use, stress response and obesity with hypertension. Consistently previous findings from our group also found that hypertensive and normotensive siblings drawn from the same families differ significantly by both degree and distribution of body fat accumulation and that genetic factors that co-segregate with hypertension appear to play a significant role in this difference.[31] Sex-specific genetic architecture has also been found to be an important mechanism underlying many complex traits; moreover, sex-specific genetic differences have been already reported for obesity, stress, substance use and hemodynamic data.[15; 29; 30; 34; 35]

Commonly associated genes driven from sex-specific and hypertension-specific analysis tended to display similar expression profiles and functional characteristics to those from general results; moreover, we found shared genes and numerous interactions among gene sets driven from general analysis and sex and hypertension-specific analysis, suggesting sex-specific and hypertension-specific genetic differences appear to be also due to variations in synaptic processes; therefore, by increasing genetic homogeneity, subgroup analysis can uncover additional components of functional modules underlying complex disorder that otherwise remain obscure following statistical adjustments; moreover, such findings can shed lights into the observed sex and hypertension differences in prevalence of these disorders. Overall these findings point to the importance of subgroup analysis in genetic studies as well as personalized medicine programs and underline the weakness of statistical adjustments.

4.6 Smoking initiation and persistence

In addition to environmental factors, smoking behavior also has genetic underpinnings; moreover, it has been reported that genetic factors contribute differently to the determination of smoking initiation and persistence in male and female smokers.[36] Consistent with these results we found that both initiation (61% in former vs. never tobacco users) and persistence of tobacco use (46% in current vs. former tobacco users) are highly attributed to genetic factors; sex-specific heritability estimates for tobacco use showed that genetic factors are more important in initiation (males = 81%, females = 56%) and persistence of tobacco use (males = 97%, females = 23%) in males compared to females; since hypertensive subjects are recommended to stop smoking therefore comparing the heritability differences of smoking behavior between smokers and non smokers seems inappropriate; however, compared to general population heritability of smoking initiation was higher in normotensive subjects while the heritability of smoking persistence was lower.

Inclusion of former smokers in this study allows us to determine whether smoking cession is associated with a similar magnitude of change to that typical of non smokers and also if the shared genetic factors between former and current tobacco users contribute to the differences. In this regard, we found several obesity related traits that their relatedness with smoking behavior seemingly are mediated by genetic factors. Thigh skinfold in whole cohort subscapular skinfold in females, superaialic skinfold and body fat percentage in normotensives along with hip thigh proximal ratio in both normotensives and hypertensives were significantly correlated with current and former tobacco users as compared to subjects with no history of tobacco use; moreover, the differences between current and former tobacco users were insignificant for these traits which suggest that the observed correlations probably roots in genetic factors that are shared between former and current tobacco users rather than smoking effect itself.

Moreover, detected SNPs following search for genotypic links between tobacco use and significantly correlated obesity, stress and hymedynamic traits with tobacco use,

highlighted the presence of genetic similarities and differences between current and former tobacco users; SNP, rs679783 was significantly associated to both smoking initiation and persistence while rs947084 was significantly associated to smoking initiation and SNP, rs36950 was significantly associated to smoking persistence.

4.7 Network based genome-wide scan

Genome-wide association studies (GWAS) armed with efficient genotyping technologies have been emerged as a major tool to identify disease susceptibility loci and have been successful detecting novel genes for several complex diseases.[37] The current GWAS have focused on single SNP analysis in which allelic frequencies of each marker are compared between affected and unaffected subjects; however, due to the factors like large multiple testing involved in these studies or the limited power of study, very few numbers of SNPs exceed the genome-wide significance threshold. The detected SNPs typically have only mild effects and account for small fraction of both the heritable component and the population disease burden; moreover, they may not be reproducible in other samples which point to presence of locus heterogeneity;[38-40] therefore, it is likely that alternative analysis approaches to GWAS data that focus on the combined effects of many loci, each making a small contribution to overall disease liability, may reveal novel insights into genetic underpinnings of complex disorders; for instance, it has been shown that any single gene polymorphism explains just 1-8% of total disease risk for a polygenic trait in studied population; however, the additive effects of several polymorphisms can be 20-70% of total genetic risk.[41]

Such approach is also supported in network-based view, namely, if a disorders is as a result of accumulating effect of several variations in a functional module producing recognizable developmental and/or physiological changes;[7; 42; 43] single SNP analysis offers limited understanding of biological mechanisms behind complex disorders; moreover, in a network-based view, locus heterogeneity appears to be inherent property of a complex

disorder since variations in different components of a network can result into the same outcome; therefore, to understand molecular mechanisms behind complex disorders; in addition, to identify the list of disease genes; understanding the detailed wiring diagram of the variations or viewing the genes in the context of biological process is important too.[44]

Previous studies also reported that common human diseases are modulated by a large number of low-risk variations which tend to localize in the functional periphery of a modular network, and these variations are not likely to be easily detectable through the use of standard single-locus-oriented univariate GWAS analysis techniques; in addition, network-based genome wide scans have already provided novel insights into the pathogenesis of polygenic disorders which are not evident in single SNP analysis approach.[7; 8; 27; 45]

Therefore, network-based genetic analysis which seeks to extract large amounts of biologically relevant information from variants that have small genetic effects; however, their joint actions will play a significant role in the development of disease, appeared to be extremely useful approach for extending current single-locus-oriented, univariate GWAS analysis techniques, such approach can compensate for the lack of statistical power due to insufficient sample size in single based GWAS. Replication of association finding at a pathway level is also much easier than replication at the SNP level. Besides, since SNPs and genes carry on their functions through intricate pathways of reactions and interaction; therefore, attempting to understand and interpret a number of significant SNPs without any unifying biological theme can be challenging and demanding.[46]

Altogether it appears that network-based approaches can help overcome the limitations imposed by univariate single-locus analysis of GWAS data and offer a powerful methodology for revealing the polygenic nature of common chronic disease susceptibility; moreover, network based approaches provide a new perspective on how large human genomic data set information can be processed to uncover pathways that otherwise would remain unrecognized; thus, beyond replication of particular SNP associations arising from

future GWAS and sequencing studies, consideration and further validation of pathway analysis may be an useful and insightful research aspect.

4.8 Disease classification and profiling

This study highlights the presence of genetic similarities among obesity, stress, substance use and hemodynamic traits. Similarly notable connections between even seemingly unrelated diseases have been observed previously, and many more will definitely be revealed by upcoming studies. For example, macular degeneration and myocardial infarction, diseases whose phenotypes appear to be unrelated, have been connected by susceptibility polymorphisms in complement factor H (CFH).[47; 48] Myocardial infarction was also connected to other inflammatory diseases, such as multiple sclerosis and rheumatoid arthritis, by MHC2TA polymorphisms;[49] P35/CDK5 signaling pathway has been implicated in both, alzheimer's disease and type 2 diabetes;[50] Several genes including ENPP1, PPARA, and FTO gene are already implicated in both obesity and diabetes;[44; 51; 52] and as mentioned earlier, Torkamnai et al[8] found shared SNPs among even seemingly unrelated traits.

The existence of intricate molecular links between sub cellular components and disease genes raises another possibility that polygenic diseases may not be as independent of one another as medical practitioners currently consider them to be and indicates that such studies can be used to indentify genetic similarities and differences among polygenic disorders and therefore may have applications for the field of nosology or disease classification. Current classification of human disease derives from observational correlation between pathological and physiological measurements with clinical syndromes. Characterizing disease in this way established a nosology that has served clinicians well to the current time. Yet, this diagnostic strategy has significant shortcomings that reflect both a lack of sensitivity in identifying preclinical disease, and a lack of specificity in defining disease unequivocally.[11; 53]

Previous genetic studies have already provided evidences of seemingly unrelated diseases are being lumped together. What were thought to be a single disease appears to root in different genotypes. Such studies point that focusing on underlying molecular signature of disorders in addition to conventional prognosis may have several benefits including; providing new insights into mechanisms of co morbidity of disorders; allowing to understand the basis of disease susceptibility and environmental effects; offering an explanation for the different phenotypic manifestations of the same disease; helping to define disease prognosis more precisely with optimal sensitivity and specificity; and eventually individualizing disease treatment for best possible therapeutic approach.[11; 53-55]

Sub-classifying histologically similar cancers by differences in surface biomarkers, transcription profiling, genetic variations or proteomic analysis is currently being applied to several malignancies, including adenocarcinoma of the breast and lymphomas, in an effort to provide better information about prognosis and response to therapy;[53; 55-57] moreover, as molecular underpinnings of many disorders were identified and the genetic similarities and differences among disorders becoming more characterized. This approach appears to eventually become more objective as an essential part of the overall diagnostic paradigm.

Considering these findings, we are aiming to classify and map our studied phenotypes by measuring the extent of genetic similarities and differences among them as well as their genetic distances with other phenotypes in our database by use of findings driven from GWAS as wells as shared heritability estimates. The constructed phenotype map can subsequently be overlaid with drug-target network to explore potential therapeutic implications.

4.9 Drug network

Identification of common molecular interface among polygenic disorders can also provides new applications for current medications; for instance, if two disorders are sharing similar modular network, perhaps drugs which are used for one, can be tested against the

other; moreover, such studies will provide further insight into possible drug side effects and contribute to development of more rational therapeutic approaches. Such Network-based thinking also raise the question whether directly targeting the product of mutated genes is efficient or targeting network properties and then accounting for indirect effects of drug is more productive; either way, a good understanding of underlying factors is essential.[58]

4.10 Conclusion

In this study, we found significant phenotypic correlations among substance use, obesity, stress and hemodynamic traits. For instance, Alcohol and tobacco users had attenuated heart rate response to mental stress; moreover, tobacco users had lower blood pressure compared to non users; Hypertensives had stronger HR and SBP response to mental stress and higher BMI compared to normotensives; Use of tobacco seemed to increase the epinephrine level in body and higher epinephrine level was correlated with lower BMI.

Consistent with phenotypic relatedness, We found shared genes associated / linked to substance use, obesity-related traits, response to mental and physical stress and hemodynamic traits including CAMK4, CNTN4, DLG2, DAG1, FHIT, GRID2, ITPR2, NOVA1, NRG3 and PRKCE forming protein interaction network, involved in synaptic plasticity and highly expressed in brain related tissues; moreover, pathway analysis on identified genes pointed ($P = 0.03$) to Long-Term Potentiation pathway, an important form of synaptic plasticity.

We found that large portions of variations of studied traits are attributed to sex and hypertension status, focusing on tobacco use we noted that even degree and the direction of correlations of obesity, hemodynamic and stress related traits with tobacco use vary according to sex and hypertension status; for instance, while in males, current tobacco users were less obese compared to never or former tobacco users, there were no such differences in females; moreover, we found several obesity related traits that their correlations with

smoking behavior seemingly root in genetic factors rather than smoking effect itself. Sex- and hypertension differences in heritabilities of many of these traits were also observed; meanwhile, specific subgroup genetic analyses uncovered additional shared synaptic genes including CAMK4, CNTN5, DNM3, KCNAB1 (Hypertension-specific), CNTN4, DNM3, FHIT, ITPR1 and NRXN3 (Sex-specific) having protein interactions with genes driven from general analysis; moreover, the results of pathway analysis and reported gene expression profiles of resultant genes from specific analyses revealed similar characteristics to those from general analysis. In addition, subgroup analysis uncovered variants inside synaptic genes, IL1RAP, PLCG1 and SCIN genes that were positively associated ($P < 0.001$) to a trait in one group and negatively associated ($P < 0.001$) to the same trait in contrary group. These findings indicate that by increasing genetic homogeneity, subgroup analysis can uncover additional components of functional modules underlying complex traits in which remain obscure following statistical adjustments; moreover, such findings can shed lights into the observed sex and hypertension differences in prevalence of these traits.

The substantial overlap among genomic determinants of substance use, stress, obesity, heart rate and blood pressure supports the notion that the genetic variations in pathways of synaptic plasticity may be a common interface behind these traits and observed sex and hypertension genetic differences. We assume the interaction between genetic make-up of synapses with environmental factors including stress influence individuals's life styles and habits; in the other side, the taken habits and lifestyle influence the body's systems including cardiovascular system and modify the cardiovascular outcomes as well as cardiovascular risk factors e.g. body weight; nonetheless, similar to different routes ended to the same location, there are other factors that influence the cardiovascular system through other mechanisms. Our results also suggest that synaptic plasticity may be a common interface behind many other complex disorders in which life style is a contributing factor, an assumption that requires further investigations.

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Appendice 1

Article1:

Table S1. List of the genes driven from general and specific genetic scans along their evidence of synaptic function

Synaptic function	Symbol[1]	Description	Type of analysis
	A2BP1	Ataxin 2-Binding Protein 1	General (commonly associated)
✓	ADAMTS3	ADAM Metallopeptidase with Thrombospondin Type 1 Motif, 3	Hypertension-specific (under linkage peak)
	ADCY8	Adenylate Cyclase 8 (Brain)	Sex-specific (commonly associated)
	AGBL4	ATP/GTP Binding Protein-Like 4	General (commonly associated)
	AGTR1	Angiotensin II Receptor, Type 1	General (under linkage peak)
✓	ANKS1B	Ankyrin Repeat and Sterile Alpha Motif Domain Containing 1B	General (commonly associated)
	ARHGAP12	Hypothetical Protein FLJ10971	Hypertension-specific (under linkage peak)
	ARHGAP18	Rho GTPase Activating Protein 18	General (commonly associated)
	ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain	Sex-specific (commonly associated)
	BACE2	Beta-Site APP-Cleaving Enzyme 2	Hypertension-specific (under linkage peak)

[1] Genes appeared in more than one set of result are underlined.

✓	BAI3	Brain-Specific Angiogenesis Inhibitor 3	Sex-specific (commonly associated)
	C14orf25	Chromosome 14 Open Reading Frame 25	Sex-specific (commonly associated)
	C14orf37	Chromosome 14 Open Reading Frame 37	Hypertension-specific (commonly associated)
	C1orf110	Chromosome 1 Open Reading Frame 110	Hypertension-specific (under linkage peak)
	C6orf191	Chromosome 6 Open Reading Frame 191	General (commonly associated)
	CADPS	Ca2+-Dependent Secretion Activator	Sex-specific (commonly associated)
✓	CAMK4	Calcium/Calmodulin-Dependent Protein Kinase Iv	General (commonly associated)
✓	CAMK4	Calcium/Calmodulin-Dependent Protein Kinase Iv	Hypertension-specific (commonly associated)
	CBLN2	Cerebellin 2 Precursor	General (under linkage peak)
	CD300LB	Cd300 Antigen Like Family Member B	Sex-specific (under linkage peak)
✓	CDH12	Cadherin 12, Type 2 (N-Cadherin 2)	Hypertension-specific (under linkage peak)
	CDH17	Cadherin 17, LI Cadherin (Liver-Intestine)	Sex-specific (commonly associated)
	CDH6	Cadherin 6, Type 2, K-Cadherin (Fetal Kidney)	Sex-specific (commonly associated)
	CHORDC1	Cysteine and Histidine-Rich Domain (Chord)-Containing 1	General (commonly associated)
✓	CHRM2	Cholinergic Receptor, Muscarinic 2	General (under linkage peak)

✓	CNTN4	Contactin 4	General (commonly associated)
✓	CNTN4	Contactin 4	Sex-specific (commonly associated)
✓	CNTN5	Neural Adhesion Molecule	Hypertension-specific (commonly associated)
	COL24A1	Collagen, Type XXIV, Alpha 1	General (under linkage peak)
	COMMD10	Comm Domain Containing 10	Hypertension-specific (under linkage peak)
	CPE	Carboxypeptidase E	Hypertension-specific (commonly associated)
✓	CPS1	Carbamoyl-Phosphate Synthetase 1, Mitochondrial	General (commonly associated)
	CSGALNACT1	Chondroitin Sulfate N-Acetylgalactosaminyltransferase 1	General (commonly associated)
	CSMD1	Cub and Sushi Multiple Domains 1	General (commonly associated)
	CSMD1	Cub and Sushi Multiple Domains 1	Hypertension-specific (commonly associated)
✓	CTNNA2	Catenin (Cadherin-Associated Protein), Alpha 2	General (commonly associated)
	CTNNA3	Catenin (Cadherin-Associated Protein), Alpha 3	Hypertension-specific (commonly associated)
✓	DAB2	Disabled Homolog 2, Mitogen-Responsive Phosphoprotein (Drosophila)	General (under linkage peak)
✓	DAG1	Dystroglycan 1 (Dystrophin-Associated Glycoprotein 1)	General (commonly associated)
✓	DCLK1	Doublecortin and Cam Kinase-Like 1	Sex-specific (commonly associated)

✓	DGKB	Diacylglycerol Kinase, Beta 90Kda	General (commonly associated)
✓	DLG2	Discs, Large Homolog 2, Chapsyn-110 (Drosophila)	General (commonly associated)
✓	DNM3	Dynamin 3	Hypertension-specific (under linkage peak)
✓	DNM3	Dynamin 3	Sex-specific (under linkage peak)
	DSCAM	Down Syndrome Cell Adhesion Molecule	Hypertension-specific (under linkage peak)
✓	DTNBP1	Dystrobrevin Binding Protein 1	Hypertension-specific (under linkage peak)
	EDEM1	ER Degradation Enhancer, Mannosidase Alpha-Like 1	General (commonly associated)
✓	ELTD1	EGF, Latrophilin and Seven Transmembrane Domain Containing 1	General (commonly associated)
✓	EPB41L3	Erythrocyte Membrane Protein Band 4.1-Like 3	General (commonly associated)
	EYS	Eyes Shut Homolog	Sex-specific (commonly associated)
	FAM134B	Hypothetical Protein Flj20152	General (commonly associated)
✓	FAM13A1	Family with Sequence Similarity 13, Member A1	General (commonly associated)
	FAM155A	Family with Sequence Similarity 155, Member A	Hypertension-specific (commonly associated)
	FAM174A	Family with Sequence Similarity 174, Member A	General (commonly associated)
	FAM190A	Family with Sequence Similarity	General (commonly associated)

		190, Member A	
	FARS2	Phenylalanine-Trna Synthetase 2 (Mitochondrial)	General (commonly associated)
✓	FHIT	Fragile Histidine Triad Gene	General (commonly associated)
✓	FHIT	Fragile Histidine Triad Gene	Sex-specific (commonly associated)
✓	GLRA3	Glycine Receptor, Alpha 3	Hypertension-specific (commonly associated)
	GPC6	Glypican 6	General (commonly associated)
✓	GRID2	Glutamate Receptor, Ionotropic, Delta 2	General (commonly associated)
✓	GRID2	Glutamate Receptor, Ionotropic, Delta 2	General (under linkage peak)
	HBEGF	Heparin-Binding Egf-Like Growth Factor	Hypertension-specific (commonly associated)
✓	HTR2A	5-Hydroxytryptamine (Serotonin) Receptor 2A	General (under linkage peak)
	IL12A	Interleukin 12A (Natural Killer Cell Stimulatory Factor 1, Cytotoxic Lymphocyte Maturation Factor 1, P35)	Sex-specific (commonly associated)
✓	IL1RAP	Interleukin 1 Receptor Accessory Protein	Hypertension-specific
✓	INHBA	Inhibin, Beta A (Activin A, Activin Ab Alpha Polypeptide)	Sex-specific (commonly associated)
✓	ITPR1	Inositol 1,4,5-Triphosphate Receptor, Type 1	Sex-specific (commonly associated)

✓	ITPR2	Inositol 1,4,5-Triphosphate Receptor, Type 2	General (commonly associated)
✓	ITPR2	Inositol 1,4,5-Triphosphate Receptor, Type 2	General (under linkage peak)
✓	KCNAB1	Potassium Voltage-Gated Channel, Shaker-Related Subfamily, Beta Member 1	Hypertension-specific (under linkage peak)
✓	KCNH1	Potassium Voltage-Gated Channel, Subfamily H (Eag-Related), Member 1	General (commonly associated)
✓	KIF5B	Kinesin Family Member 5B	Hypertension-specific (under linkage peak)
	KRT12	Keratin 12 (Meesmann Corneal Dystrophy)	General (commonly associated)
	KSR2	Kinase Suppressor Of Ras 2	Sex-specific (commonly associated)
	L3MBTL4	L(3)Mbt-Like 4 (Drosophila)	General (commonly associated)
✓	LPHN2	Latrophilin 2	General (under linkage peak)
	LRP1B	Low Density Lipoprotein-Related Protein 1B (Deleted In Tumors)	General (commonly associated)
	LRP1B	Low Density Lipoprotein-Related Protein 1B (Deleted In Tumors)	General (under linkage peak)
	LRRIQ1	Leucine-Rich Repeats and IQ Motif Containing 1	Sex-specific (commonly associated)
	MACROD2	Chromosome 20 Open Reading Frame 133	General (commonly associated)
	MAN1A1	Mannosidase, Alpha, Class 1A, Member 1	Hypertension-specific (commonly associated)

	MDM1	Hypothetical Protein Loc54104	General (commonly associated)
	MTPN	Myotrophin	General (under linkage peak)
	MYLIP	Myosin Regulatory Light Chain Interacting Protein	Hypertension-specific (under linkage peak)
✓	MYO10	Myosin X	General (commonly associated)
✓	NETO1	Neuropilin (NRP) and Tolloid (TLL)-Like 1	General (under linkage peak)
	NKAIN2	T-Cell Lymphoma Breakpoint Associated Target 1	Hypertension-specific (commonly associated)
✓	NLGN1	Neuroligin 1	Sex-specific (commonly associated)
✓	NOVA1	Neuro-Oncological Ventral Antigen 1	General (commonly associated)
✓	NR4A2	Nuclear Receptor Subfamily 4, Group A, Member 2	General (commonly associated)
✓	NRG3	Neuregulin 3	General (commonly associated)
✓	NRXN3	Neurexin 3	Sex-specific (commonly associated)
	ODF2L	Outer Dense Fiber Of Sperm Tails 2-Like	General (under linkage peak)
	ODZ2	Odz, odd Oz/Ten-M Homolog 2 (Drosophila)	General (commonly associated)
	ODZ3	Odz, odd Oz/Ten-M Homolog 3 (Drosophila)	General (commonly associated)
✓	ODZ4	Odz, odd Oz/Ten-M Homolog 4 (Drosophila)	General (commonly associated)

	OLFM4	Olfactomedin 4	General (commonly associated)
	OLFM4	Olfactomedin 4	Sex-specific (under linkage peak)
	PAPPA2	Pappalysin 2	Hypertension-specific (under linkage peak)
	PCDH18	Protocadherin 18	Sex-specific (commonly associated)
	PCDH9	Protocadherin 9	Sex-specific (commonly associated)
	PCM1	Pericentriolar Material 1	General (commonly associated)
	PCM1	Pericentriolar Material 1	General (under linkage peak)
	PDP1	Pyruvate Dehyrogenase Phosphatase Catalytic Subunit 1	Sex-specific (commonly associated)
	PFDN1	Prefoldin Subunit 1	Hypertension-specific (commonly associated)
	PHLDB2	Pleckstrin Homology-Like Domain, Family B, Member 2	General (commonly associated)
✓	PKIA	Protein Kinase (Camp-Dependent, Catalytic) Inhibitor Alpha	General (commonly associated)
✓	PLCG2	Phospholipase C, Gamma 2 (Phosphatidylinositol-Specific)	Sex-specific
	PRDM9	PR Domain Containing 9	Hypertension-specific (under linkage peak)
✓	PRKCE	Protein Kinase C, Epsilon	General (commonly associated)
	PRR20	Proline Rich 20A	General (under linkage peak)
	PRRX1	Paired Related Homeobox 1	Hypertension-specific (under

			linkage peak)
	PTGER4	Prostaglandin E Receptor 4 (subtype EP4)	General (under linkage peak)
✓	PTPRD	Protein Tyrosine Phosphatase, Receptor Type, D	General (commonly associated)
✓	PTPRD	Protein Tyrosine Phosphatase, Receptor Type, D	Hypertension-specific (commonly associated)
	PTPRM	Protein Tyrosine Phosphatase, Receptor Type, M	General (commonly associated)
✓	PTPRR	Protein Tyrosine Phosphatase, Receptor Type, R	Sex-specific (commonly associated)
	PVT1	Pvt1 Oncogene Homolog, Myc Activator (Mouse)	Sex-specific (commonly associated)
	PXMP3	Peroxisomal Membrane Protein 3, 35Kda (Zellweger Syndrome)	General (commonly associated)
✓	RAP1B	RAP1B, Member Of Ras Oncogene Family	General (commonly associated)
✓	RGS4	Regulator of G-Protein Signalling 4	Hypertension-specific (under linkage peak)
	RIT2	Ras-Like Without Caax 2	General (commonly associated)
	RMI1	Chromosome 9 Open Reading Frame 76	General (commonly associated)
	RORA	RAR-Related Orphan Receptor A	General (commonly associated)
	RORA	RAR-Related Orphan Receptor A	Hypertension-specific (commonly associated)
	RORA	RAR-Related Orphan Receptor A	Hypertension-specific (under linkage peak)

✓	SCIN	Scinderin	Sex-specific (commonly associated)
✓	SCIN	Scinderin	Sex-specific
	SDK1	Sidekick Homolog 1 (Chicken)	Hypertension-specific (commonly associated)
✓	SEMA6A	Ht018 Protein	Hypertension-specific (under linkage peak)
	SGCG	Sarcoglycan, Gamma (35Kda Dystrophin-Associated Glycoprotein)	Sex-specific (commonly associated)
✓	SGCZ	Sarcoglycan Zeta	General (commonly associated)
	SLC25A21	Oxodicarboxylate Carrier	Sex-specific (commonly associated)
	SLC28A3	Solute Carrier Family 28 (Sodium-Coupled Nucleoside Transporter), Member 3	General (commonly associated)
	SLCO4C1	Hypothetical Protein Pro2176	General (commonly associated)
✓	SNCAIP	Synuclein, Alpha Interacting Protein (Synphilin)	General (commonly associated)
	SNX2	Sorting Nexin 2	General (commonly associated)
	SOX6	SRY (Sex Determining Region Y)-Box 6	Hypertension-specific (under linkage peak)
✓	SPINK5	Serine Peptidase Inhibitor, Kazal Type 5	Hypertension-specific (commonly associated)
	ST8SIA4	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 4	General (commonly associated)
	STRN3	Striatin, Calmodulin Binding	Sex-specific (commonly

		Protein 3	associated)
	SYT17	Synaptotagmin XVII	Hypertension-specific (under linkage peak)
✓	SYT4	Synaptotagmin IV	General (commonly associated)
	TDRD3	Tudor Domain Containing 3	General (commonly associated)
	TLL1	Tolloid-Like 1	General (under linkage peak)
	TMC5	Transmembrane Channel-Like 5	Hypertension-specific (under linkage peak)
	TMEM99	Transmembrane Protein 99	General (commonly associated)
	TTLL7	Tubulin Tyrosine Ligase-Like Family, Member 7	General (under linkage peak)
	UBR4	Zinc Finger, Ubr1 Type 1	General (commonly associated)
	ZNF532	Zinc Finger Protein 532	Hypertension-specific (commonly associated)

Article2:

Table S1. Descriptive statistics of obesity, stress and hemodynamic related traits distributed by sex and hypertension status along with their correlations[1] with sex, age[2] and hypertension[3]

Trait	Females				Males				Total		Sex (females)		Aging		Hypertension (hypertensives)	
	Norm		Hyper		Norm		Hyper									
	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	Z	P	Z	P	Z	P
BMI (kg/m2)	175	24.7±0.4	254	27.5±0.3	162	26.3±0.4	201	28.1±0.3	792	26.8±0.2	-4.9	**5.E-07**	2.7	**4.E-03**	5.4	**3.E-08**
Waist Hip ratio	171	0.8±0	251	0.8±0	161	0.9±0	202	1±0	785	0.9±0	-24.4	**7.E-132**	8.8	**5.E-19**	4.5	**3.E-06**
Waist circumference (cm)	172	80.1±1	251	87.7±0.9	161	92.9±0.9	203	99.3±0.8	787	90.1±0.5	-16	**1.E-57**	5.3	**6.E-08**	4.8	**1.E-06**
Hip circumference (cm)	171	99.2±0.8	251	102.9±0.7	161	98±0.5	202	100.8±0.5	785	100.6±0.3	2.1	**2.E-02**	1	2.E-01	3.8	**7.E-05**
Hip Thigh Proximal ratio	171	1.7±0	238	1.7±0	158	1.7±0	193	1.7±0	760	1.7±0	-4	**3.E-05**	15.5	**1.E-54**	-0.6	3.E-01
Thigh proximal circumference (cm)	171	59±0.6	238	58.8±0.4	159	57.2±0.5	194	58.1±0.4	762	58.3±0.2	4.1	**2.E-05**	-5.5	**2.E-08**	3	**2.E-03**
Thigh mid circumference (cm)	171	52.3±0.5	241	52.8±0.4	159	52.7±0.5	195	53.4±0.4	766	52.8±0.2	-0.8	2.E-01	-4.6	**2.E-06**	4.1	**2.E-05**
Thigh distal circumference (cm)	171	39.8±0.4	239	40.6±0.3	159	40±0.3	194	40.9±0.3	763	40.4±0.2	-0.8	2.E-01	-1.6	6.E-02	3.2	**7.E-04**
Skinfold bicep1 (mm)	160	21.9±1.1	202	23.7±0.8	154	16.4±1	180	16.8±1	696	19.9±0.5	6.2	**2.E-10**	1.2	1.E-01	4.4	**7.E-06**
Skinfold bicep2 (mm)	160	22.1±1.1	202	23.9±0.8	154	16.7±1	180	16.9±1	696	20.1±0.5	6	**1.E-09**	1.1	1.E-01	4.3	**7.E-06**
Skinfold bicep3 (mm)	160	22.3±1.1	201	24±0.9	154	16.7±1	180	16.8±1	695	20.1±0.5	5.9	**2.E-09**	1	2.E-01	4	**3.E-05**
Mean skinfold biceps (mm)	160	22.1±1.1	202	23.9±0.8	154	16.6±1	180	16.8±1	696	20±0.5	6	**7.E-10**	1.1	1.E-01	4.3	**1.E-05**
Skinfold triceps1 (mm)	161	30±1	202	34.1±0.8	154	23±1	180	23.8±1	697	28±0.5	8	**9.E-16**	0.9	2.E-01	4.1	**2.E-05**
Skinfold triceps2 (mm)	161	30.2±1	202	34.5±0.8	154	23.3±1.1	179	23.6±0.9	696	28.2±0.5	7.7	**6.E-15**	1	2.E-01	4.2	**1.E-05**
Skinfold triceps3 (mm)	161	30.4±1	201	34.5±0.8	154	23.2±1.1	179	23.6±1	695	28.2±0.5	7.7	**9.E-15**	1	2.E-01	4.1	**2.E-05**

[1] Correlation test was performed using GEE. The sign of Z (Z-score) shows the direction of correlation, the positive Z means a positive correlation and vice versa.
[2] Correlation model is trait ~ sex + age.
[3] Correlation model is hypertension status ~ sex + age + substance use + trait.

Mean skinfold triceps (mm)	161	30.2±1	202	34.4±0.8	154	23.1±1.1	180	23.8±1	697	28.2±0.5	7.7	**5.E-15**	1	2.E-01	4.2	**1.E-05**
Skinfold subscapular1 (mm)	161	24±1	201	28.1±0.8	154	22.8±0.8	177	25.9±0.8	693	25.4±0.4	2.2	**2.E-02**	1.3	1.E-01	4.1	**2.E-05**
Skinfold subscapular2 (mm)	161	24.3±1	201	28.2±0.9	154	22.7±0.8	177	25.7±0.8	693	25.5±0.4	2.3	**1.E-02**	1.2	1.E-01	4	**4.E-05**
Skinfold subscapular3 (mm)	161	24.4±1	201	28.3±0.9	154	22.9±0.9	177	25.8±0.8	693	25.5±0.5	2.3	**1.E-02**	1.1	1.E-01	4	**3.E-05**
Mean skinfold subscapular (mm)	161	24.2±1	201	28.2±0.9	154	22.8±0.8	177	25.8±0.8	693	25.5±0.4	2.2	**1.E-02**	1.2	1.E-01	4	**3.E-05**
Skinfold suprailiac1 (mm)	159	23.7±1.1	201	26.9±0.7	153	21.6±1	177	24.8±0.9	690	24.5±0.5	3.1	**9.E-04**	-1	2.E-01	3.8	**7.E-05**
Skinfold suprailiac2 (mm)	159	23.7±1.1	201	27.2±0.7	153	21.9±1	177	24.8±0.9	690	24.6±0.5	3.2	**8.E-04**	-1	2.E-01	4.1	**2.E-05**
Skinfold suprailiac3 (mm)	159	23.8±1.1	201	27.2±0.7	153	21.9±1	177	25.1±0.9	690	24.7±0.5	2.9	**2.E-03**	-0.9	2.E-01	4.1	**2.E-05**
Mean skinfold suprailiac (mm)	159	23.7±1.1	201	27.1±0.7	153	21.8±1	177	24.9±0.9	690	24.6±0.5	3	**1.E-03**	-0.9	2.E-01	4	**3.E-05**
Skinfold thigh1 (mm)	145	39.2±1.1	191	43±0.9	149	26.9±1.3	175	24.3±1.1	660	33.5±0.6	13.2	**4.E-40**	-1.1	1.E-01	2.5	**6.E-03**
Skinfold thigh2 (mm)	144	39.2±1.1	191	43.3±0.9	149	27.1±1.3	175	24.5±1.1	659	33.7±0.6	12.3	**4.E-35**	-1	2.E-01	2.5	**6.E-03**
Skinfold thigh3 (mm)	144	39.5±1.1	191	43.6±0.9	148	27±1.3	175	24.6±1.1	658	33.9±0.6	13.4	**3.E-41**	-1.2	1.E-01	2.7	**4.E-03**
Mean skinfold thigh (mm)	145	39.4±1.1	191	43.3±0.9	149	27.1±1.3	175	24.5±1.1	660	33.8±0.6	12.9	**2.E-38**	-1.1	1.E-01	2.6	**5.E-03**
Body fat percentage bioimpedance	137	30.4±0.8	177	37.4±0.8	128	21.2±0.7	158	24.6±0.6	600	28.9±0.4	18.7	**5.E-78**	9.7	**1.E-22**	3.5	**2.E-04**
Body fat (%)	158	36.3±0.6	201	39.1±0.4	153	25±0.5	175	26±0.4	687	32±0.3	27.7	**3.E-169**	1	2.E-01	4.4	**5.E-06**
Supine EP (pg/ml)	66	41.9±2.1	40	40.5±2.6	57	43.3±2.3	49	46±3.2	212	42.9±1.3	-1.5	7.E-02	0.5	3.E-01	0	5.E-01
Standing EP (pg/ml)	68	50.5±3.3	37	38.6±2.4	54	57±4.7	52	54.9±3.7	211	51.2±1.9	-3.1	**1.E-03**	-2.6	**5.E-03**	-0.7	2.E-01
Response EP (pg/ml)	63	9.6±3.6	31	-1.1±2.8	54	13.8±4.6	43	6±4	191	8.2±2	-1	2.E-01	-2.4	**9.E-03**	-1.2	1.E-01
Supine NE (pg/ml)	73	171.7±7.1	48	167.3±9.8	57	169.4±8.6	60	160.2±9.5	238	167.4±4.3	0.7	2.E-01	-0.2	4.E-01	-0.9	2.E-01
Standing NE (pg/ml)	73	391.8±14.9	48	407.9±24.6	55	397.1±23.3	61	395.9±20.7	237	397.4±10.1	0.4	4.E-01	1.6	5.E-02	0.2	4.E-01
Response NE (pg/ml)	73	220.1±11.2	48	240.6±18.8	55	230.3±20.5	60	235.8±15.2	236	230.7±8	0	5.E-01	2.1	**2.E-02**	0.6	3.E-01
DBP response to mental stress (mmHg)	77	10±0.8	57	12.1±1	64	9.6±0.9	71	9.4±0.9	269	10.2±0.4	1.5	7.E-02	-1.3	1.E-01	1.4	8.E-02
DBP recovery mental stress (mmHg)	77	10.5±0.8	57	13.9±1	64	10.5±0.9	71	9.9±0.8	269	11.1±0.5	1.4	8.E-02	-0.9	2.E-01	1.5	6.E-02
Delta DBP (mmHg)	77	0.4±0.4	57	1.7±0.6	64	0.9±0.4	71	0.5±0.5	269	0.8±0.2	0.6	3.E-01	0.9	2.E-01	0.1	5.E-01
SBP response to mental stress (mmHg)	77	12.1±1.3	56	18.4±1.6	64	14.9±1.4	71	19.5±1.6	268	16.1±0.7	-1.5	7.E-02	0.9	2.E-01	2	**2.E-02**

SBP recovery from mental stress (mmHg)	77	19.2±1.3	56	30.1±1.5	64	22.1±1.4	71	28.5±1.6	268	24.7±0.7	-0.9	2.E-01	2.6	**4.E-03**	3	**1.E-03**
Delta SBP (mmHg)	77	7.1±0.6	56	11.8±1	64	7.2±0.7	71	9±0.9	268	8.6±0.4	1.7	**4.E-02**	3.5	**2.E-04**	1.9	**3.E-02**
HR response to mental stress (beast/min)	78	7.8±1.4	57	9.4±1.1	64	7±1.1	71	8.5±1	270	8.1±0.6	0.7	2.E-01	-0.6	3.E-01	1.8	**3.E-02**
HR recovery from mental stress (beast/min)	78	8.8±1.4	57	10.4±1.1	64	6.7±1.2	71	8.5±1	270	8.6±0.6	1.7	**4.E-02**	-0.4	3.E-01	1.5	7.E-02
Delta HR (mmHg)	78	1±0.5	57	1.1±0.6	64	-0.3±0.4	71	0±0.5	270	0.4±0.2	2.3	**1.E-02**	0.2	4.E-01	-0.2	4.E-01
Average sitting DBP (mmHg)	77	69.2±0.9	57	79±1.4	64	75.1±1.1	69	86±1.3	267	77.1±0.7	-5.1	**2.E-07**	6.8	**4.E-12**	4.1	**2.E-05**
Average sitting SBP (mmHg)	77	109±1.3	57	136.8±2.6	64	118.1±1.3	69	136±2.1	267	124.1±1.2	-2.6	**4.E-03**	6.2	**3.E-10**	5.8	**4.E-09**
Average sitting HR (beast/min)	77	71.1±1.2	57	73.8±1.5	64	64.7±1.2	67	67.1±1.2	265	69.1±0.7	4.4	**6.E-06**	-1.5	6.E-02	2.1	**2.E-02**
Mean ambulatory DBP (mmHg)	61	67.4±0.8	43	79.3±1.5	48	73.3±1.1	51	84.7±1.2	203	75.6±0.7	-6.2	**3.E-10**	9.3	**5.E-21**	5.9	**2.E-09**
Mean ambulatory SBP (mmHg)	61	110.3±1	43	130.6±2.4	48	120.7±1.3	51	133.7±1.9	203	122.9±1	-5.3	**8.E-08**	5.6	**9.E-09**	5.4	**3.E-08**
Mean ambulatory HR (mmHg)	61	74.9±1	43	79.9±1.4	48	70.5±1.6	51	76.3±1.5	203	75.3±0.7	1.7	**4.E-02**	0.3	4.E-01	2.9	**2.E-03**
Sleep DBP (mmHg)	74	61.4±1	50	70.3±1.6	61	67.2±1.1	63	74.8±1.3	248	68±0.7	-5.8	**3.E-09**	5.5	**2.E-08**	3.8	**8.E-05**
Sleep SBP (mmHg)	74	102.5±1.1	50	117.6±2.4	61	112.6±1.2	63	122.2±2	248	113±1	-5.5	**2.E-08**	4.1	**2.E-05**	4.3	**1.E-05**
Sleep HR (beast/min)	74	71.2±1.2	49	74.8±1.4	60	65.8±1.3	63	67.3±1.6	246	69.6±0.7	3.4	**4.E-04**	0.3	4.E-01	1.2	1.E-01
Sleep MAP (mmHg)	74	75.1±1	50	86.1±1.8	61	82.3±1	63	90.6±1.4	248	83±0.7	-5.9	**2.E-09**	5	**2.E-07**	4.2	**1.E-05**
Sleep PP (mmHg)	74	41.1±0.7	50	47.4±1.6	61	45.4±0.9	63	47.4±1.4	248	45±0.6	-2.5	**6.E-03**	0.7	2.E-01	3.1	**9.E-04**
Wake DBP (mmHg)	73	71±0.9	49	79.6±1.6	62	75.5±1	63	84.4±1.3	247	77.2±0.7	-5	**3.E-07**	5.5	**2.E-08**	3.8	**8.E-05**
Wake SBP (mmHg)	73	115.3±1.2	49	131.3±2.5	62	124.4±1.3	63	136.5±1.9	247	126.2±1	-5.4	**4.E-08**	4.7	**1.E-06**	4.5	**4.E-06**
Wake HR (beast/min)	73	81.6±1	49	85±1.6	62	76.6±1.4	63	81.7±1.8	247	81±0.8	2.1	**2.E-02**	0.6	3.E-01	2.2	**1.E-02**
Wake MAP (mmHg)	73	85.8±0.9	49	96.8±1.7	62	91.8±1	63	101.8±1.4	247	93.6±0.7	-5.9	**2.E-09**	5.6	**1.E-08**	4.5	**4.E-06**
Wake PP (mmHg)	73	44.3±0.9	49	51.8±1.7	62	48.9±1	63	52.1±1.4	247	48.9±0.6	-2.8	**2.E-03**	1.6	6.E-02	2.9	**2.E-03**
Overall PP (mmHg)	61	42.9±0.7	43	51.3±1.6	48	47.4±0.9	51	48.9±1.2	203	47.3±0.6	-2.2	**1.E-02**	0.5	3.E-01	3.2	**7.E-04**